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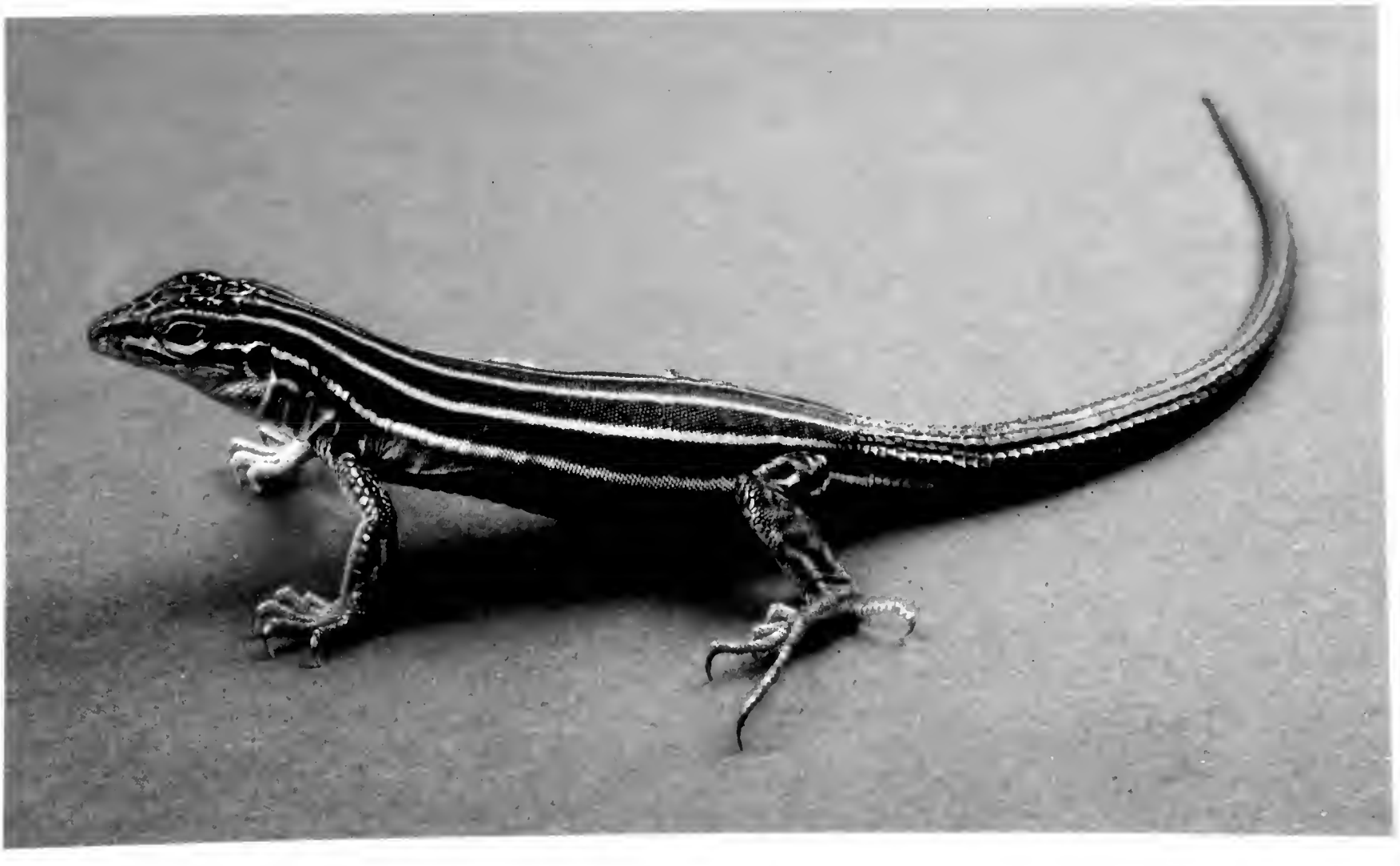
# The Second Known Tetraploid Species of Parthenogenetic Tetrapod (Reptilia: Squamata: Teiidae): Description, Reproduction, Comparisons with Ancestral Taxa, and Origins of Multiple Clones

Charles J. Cole, Harry L. Taylor, William B. Neaves, Diana P. Baumann, Aracely Newton, Robert Schnittker, and Peter Baumann



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# THE SECOND KNOWN TETRAPLOID SPECIES OF PARTHENOGENETIC TETRAPOD (REPTILIA: SQUAMATA: TEIIDAE): DESCRIPTION, REPRODUCTION, COMPARISONS WITH ANCESTRAL TAXA, AND ORIGINS OF MULTIPLE CLONES

CHARLES J. COLE,[1] HARRY L. TAYLOR,[2] WILLIAM B. NEAVES,[3] DIANA P. BAUMANN,[3] ARACELY NEWTON,[3,4] ROBERT SCHNITTKER,[3] AND PETER BAUMANN[5]

## CONTENTS



[1] Division of Vertebrates (Herpetology), American Museum of Natural History, 200 Central Park West, New York, New York 10024. Author for correspondence (cole@amnh.org).

[2] Department of Biology, Regis University, Denver, Colorado 80221.

[3] Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, Missouri 64110.

[4] Present address: Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, BioLabs Building 2077, Cambridge, Massachusetts 02138.

[5] Howard Hughes Medical Institute and Stowers Institute for Medical Research, Kansas University Medical Center, Kansas City, Missouri 64110.

ABSTRACT. We describe the second known tetraploid amniote that reproduces by parthenogenetic cloning. This all-female species of whiptail lizard originated in the laboratory from hybridization between *Aspidoscelis uniparens* (triploid parthenogen) and *Aspidoscelis inornatus* (diploid bisexual species). Similar clonal lineages of tetraploids arose from at least 44 $F_1$ hybrid females. These were produced by at least 15 *A. uniparens* that mated with several different males of *A. inornatus* from both New Mexico and Arizona stock.

Inheritance of alleles at eight microsatellite deoxyribonucleic acid (msDNA) loci in the tetraploid species confirms its parentage, whereas DNA quantification and behavior of chromosomes in meiosis demonstrate that tetraploidy and heterozygosity are maintained generation after generation.

We compared univariate and multivariate variation in scalation between the tetraploids, their parental taxa, and four museum specimens from New Mexico that were reported as putative hybrids. Two of the putative hybrids are confirmed as such, but the other two are *A. inornatus*. The similarities of *A. uniparens* and the tetraploids suggest that tetraploid females may exist in old samples misidentified as *A. uniparens*. Clones of the tetraploid species are so similar to each other in morphology and msDNA that we have been unable to distinguish most individuals or separate lineages from the $P_1$ through multiple generations (up to $F_7$) on the basis of the 12 msDNA loci analyzed in this study.

We discuss taxonomic aspects of the multiple hybrid origins of similar clones and apply one specific name. For some systematists, depending on the species concept preferred, these lizards represent a complex of multiple cryptic species that cannot be reliably identified or diagnosed (one species for each $F_1$ hybrid female that cloned a lineage). These specimens of known parentage provide valuable insights for taxonomic treatment of natural parthenogenetic clones. In addition, we show that the degree of morphological variation in clonal parthenogens and bisexual *A. inornatus* can be similar to each other.

Key words: *Aspidoscelis priscillae*, New species, Tetraploids, Parthenogenesis, Hybrid origins, Reproduction, Clonal lineages, Variation, Lizards

## INTRODUCTION

The parthenogenetic species of whiptail lizards (*Aspidoscelis*) consist of all-female clones that initially had hybrid origins generations ago, as reviewed by Reeder et al. (2002) and Cole et al. (2014). Diagnostic lineages are formally recognized as species because of their unique ancestry, which may include diploid, triploid, or tetraploid lineages. This report is a follow-up to the description of the first known tetraploid tetrapod (Cole et al., 2014). The new tetraploid is also a product of hybridization in the laboratory, although similar specimens are known from nature. Providing a name is essential for future unambiguous communications, as these organisms are used for molecular research on basic biological processes at the cellular and subcellular levels (e.g., Lutes et al., 2010, 2011; Newton et al., 2016), and sequence data are being generated and deposited with online data information services (e.g., GenBank, RefSeq, UniProt).

Laboratory hybridization of whiptail lizards at Stowers Institute, which involves crosses among sexual species and among sexual and parthenogenetic species, has produced diploid, triploid, and tetraploid offspring, depending on the species crossed. Some of the hybrids have been founders of multiple self-sustaining clonal lineages. In contrast, crosses among other species have yet to yield reproducing lineages. Continuing research is expected to provide insights into the pivotal role of interspecific hybridization in the origin of parthenogenesis in whiptail lizards, the proliferation of unisexual species, and some of the molecular causes of successful parthenogenesis versus hybrid sterility.

In this paper we describe another unique unisexual species of hybrid origin, which clones itself parthenogenetically and is reproductively isolated from all other species. Tetraploid clonal lineages (up to the $F_7$ generation to date) were produced after hybridization of females of *Aspidoscelis uniparens* (a triploid parthenogen) with males of *Aspidoscelis inornatus* (a diploid gonochoristic or bisexual species). Very similar tetraploid lineages were produced by hybridization between the parental forms from both New Mexico (NM) and Arizona (AZ) stock, from localities more than 300 km (linear) apart. We provide genetic

evidence that the tetraploids are clonal parthenogens, and we compare univariate and multivariate morphological variation among the tetraploids and their parental ancestral taxa. We also show that the relative extent of morphological variation in the clonal species can be equivalent to that of the gonochoristic *A. inornatus*.

About 50 years ago, four possible hybrids of apparently the same hybrid combination of *A. uniparens* × *A. inornatus* were found at two localities near Nutt and Socorro, NM (Wright, 1968). We examined these specimens and clarified their identities by a multivariate analysis of morphological characters.

## MATERIALS AND METHODS

### Species, Populations Sampled, Specimens, and Museum Abbreviations for Specimens Examined

We examined specimens of the triploid parthenogen *A. uniparens*; diploid bisexual (gonochoristic) *A. inornatus*; laboratory $F_1$ hybrids of *A. uniparens* × *A. inornatus*; the new species, composed of clones of multiple $F_1$ hybrids representing multiple generations; and the four putative hybrids collected in the field many years ago (see Appendix 1, Specimens Examined). For species of *Aspidoscelis*, we followed Tucker et al. (2016) in using the *...us* ending on specific epithets that formerly ended with *...a*.

*Aspidoscelis inornatus* warrants additional discussion here, as we used population samples from both NM and Cochise County, AZ. Past references relegating our samples to different subspecies or different species (e.g., *Aspidoscelis arizonae* for ones from Cochise County) have been found to lack support (Cole et al., 2010; Sullivan et al., 2013, 2014). Consequently, we refer to our two population samples of *A. inornatus* specifically as those from NM stock or from AZ stock. We also have separate samples of *A. uniparens* from NM and AZ stocks, with the former treated as a pooled sample from two sites in NM (see below and Appendix 1).

Methods for maintaining *Aspidoscelis* in a captive colony were those used by Lutes et al. (2010, 2011) and Jewell et al. (2015). Identity of individual lizards is tracked as follows: whenever possible, the individual mother of each egg clutch is noted (as is the father, if any); hatchlings are noted as to specific clutch; and throughout life individuals are photographed periodically and tracked as to which enclosures they occupy at any time. Whenever possible, lineage membership is confirmed using microsatellite deoxyribonucleic acid (msDNA) analysis (e.g., Lutes et al., 2011). However, on many occasions a clutch is laid in an enclosure containing several females, so individual identity of the mother may not be clear. Also, for many of the individual adults and offspring of the species involved in this paper, msDNA was so similar from lizard to lizard that individual parents or lineages could not be specified with confidence using the 12 markers routinely used with these specimens.

In the extensive laboratory records, first-generation hybrids are referred to as representing the $H_1$ generation. Their cloned offspring are the $H_2$ generation, the next is the $H_3$ generation, and so on. However, in this paper we use a modified reference to the generations, to emphasize both the origin of the new species and the fact that individuals of cloned generations are not hybrids. Herein, first-generation hybrids are referred to as $F_1$ hybrids. The next generation, cloned by $F_1$ females, is the $P_1$ generation (parental) of the new species. The next generation, cloned by $P_1$ females, is the $F_1$ generation (filial) of the new species, and so on. Consequently, our reference to the $F_1$ generation of the new species is equivalent to the $H_3$ generation as noted in the laboratory records, and so on.

All specimens examined and localities sampled are listed in Appendix 1. The specimens are in the following collections: American Museum of Natural History (AMNH), New York; Museum of Comparative Zoology (MCZ), Harvard University,

TABLE 1. OLIGONUCLEOTIDE PRIMERS USED FOR AMPLIFICATION OF MICROSATELLITE (MS) DNA SEQUENCES.

| MS Locus | Primer | Sequence |
|---|---|---|
| Ai5013 | Holi528T | AATTAATGTGCAGCACTAT |
| | Holi534B | GGCAGTTTTTCAGCTAAG |
| Cvanμ24 | Holi340T | TTTAATGCATCCACTGAGTC |
| | Holi341B | GGAATATAGTGGCATATCAG |
| MS15 | Holi483T | TTAAAGCAGAGGTCAGGTTATC |
| | Holi507B | GATGGAAGAATAGGATGATGAA |
| MS12 | Holi486T | TACCCACCTGGAGATGTTTAG |
| | Holi510B | AGGACGCCTTAAAATAGGAAG |
| MS14 | Holi482T | TGGAGGCAGTCTTGGTATC |
| | Holi506B | GAACATTGACCGCATCAC |
| MS1 | Holi346T | TGCATGATGGAGGAATCTTC |
| | Holi347B | CTAGTGGTGATAGAAACATGG |
| MS7 | Holi100T | AACTAAGTGCTAAGTGTGAC |
| | Holi101B | ACAGTCTTAGAGATCACAAG |
| MS8 | Holi181T | ACACCCAAAGTCCTCAACAG |
| | Holi182B | CTAGTACATGTGTAAGGGTG |

Cambridge, Massachusetts; Museum of Southwestern Biology (MSB), University of New Mexico, Albuquerque; and Stowers Institute for Medical Research (SIMR), Kansas City, Missouri.

### Karyotype, msDNA, DNA Quantification, Reproduction, and Chromosome Pairing in Meiosis

Chromosomes were prepared for study by culturing fibroblasts from three embryos following the methods used by Lutes et al. (2011). Ten typical metaphase spreads were studied from each embryo.

Microsatellite DNA analysis was performed as previously described (Lutes et al., 2011), except that the amount of sample used for each polymerase chain reaction was increased to 1.5 μl. Primers used for each microsatellite are listed in Table 1. Collection of whole blood and quantification of DNA content by fluorescence-assisted cell sorting (FACS) were performed as by Lutes et al. (2011) with the following modifications: after collection of cells and decanting the supernatant, the cells were resuspended in citrate buffer at a density of $2.5 \times 10^6$ cells/ml. Two hundred microliters of cell suspension were transferred into a 5-ml polypropylene round-bottom tube (Falcon #352063) and incubated with 450 μl of 30 mg/l trypsin, pH 7.6 (T-0134, Sigma), diluted in buffer S (3.4 mM trisodium citrate, 0.1% Triton X-100, 1.5 mM spermine, and 0.38 mM Tris HCl) for 10 minutes with gentle rotation, followed by the addition of 375 μl of trypsin inhibitor solution (0.5 mg/ml trypsin inhibitor, T9003, Sigma, 0.1 mg/ml RNase A, R-5500, Sigma prepared in buffer S) for an additional 10 minutes with gentle rotation. Staining solution was prepared (0.42 mg/ml propidium iodide [P-4170, Sigma] and 3.33 mM spermine in buffer S) and 375 μl were added to the cell suspension and incubated with gentle agitation for 10 minutes while protected from light. Events were collected with a MACSQuant analyzer (Miltenyi Biotec) by excitation at 488 nm and collection at 565–605 nm with a threshold set to exclude small debris. No gates were used. Isolation of germinal vesicles and acquisition of image stacks by confocal microscopy were performed as described in Lutes et al. (2010).

### Morphological Characters and Statistical Analyses

The univariate characters are listed in Appendix 2. Nomenclature for epidermal scales follows Smith (1946). Sex was deter-

mined by dissection and examination of primary sex characters, a history of oviposition, or examination of relevant external secondary characters.

We used NCSS© software for data screening, statistical routines and tests, and scatter plot construction. We used a database of 466 specimens (Appendix 1) to address questions concerning patterns of morphological variation among and between particular samples. However, principal components analysis (PCA) and canonical variate analysis (CVA) disregard specimens with incomplete data, so specimens with damaged or missing characters could not be included in the multivariate statistical analyses. We provide the definitive sample sizes in the appropriate tables and list the specimens in Appendix 1.

Consistent with analyses associated with describing the parthenogenetic tetraploid *Aspidoscelis neavesi* (Cole et al., 2014), we used PCAs of 10 univariate meristic characters (Appendix 2) to summarize variation in the context of single, pooled samples of individuals unassigned to any group. The PC scores for individual specimens are linear compounds of original meristic character scores and coefficients factored from a correlation matrix. After a PCA, the variation in the pooled sample is now expressed by uncorrelated PCs, so that most of the variation is represented by PC1, with decreasing proportions of the variation found in a PC1 through PC10 sequence. We used the PCs in two ways. First, because most of the variation is concentrated in the first few PCs, we used PC1 and PC2 to interpret relative morphological variation among the groups included in the PCA. Second, we used the 10 PCs from each analysis as characters in a follow-up CVA to produce new characters, (CVs ), that would determine how well the samples included in the PCA could be discriminated or distinguished from each other. The groups classified a priori for the CVAs were samples of different taxa or $F_1$ hybrids, as specified in the appropriate discussions and tables. The PCs to include in a CVA were determined by stepwise selection of PCs—those that increased intergroup separation at each step. A CVA model retained PCs with $F$-to-enter probabilities $< 0.05$ if the probability did not exceed 0.06 with the addition of other components. Jombart et al. (2010) detailed the general benefits of a coupled PCA-CVA procedure, and Taylor and Walker (2014), Taylor et al. (2015), and Cole et al. (2016) described benefits specifically related to studies of morphological variation in several species of *Aspidoscelis*.

We used a $T^2$ test, $\alpha = 0.05$, to check each sample for the presence of multivariate outliers, $t$ tests to test means of two independent samples, and one-way analyses of variance (ANOVAs) for three or more samples. We used Welch's test of means as an alternative to $F$ tests to allow for unequal variances (Welch, 1951; NCSS 11 Statistical Software, 2016, Kaysville, Utah). We followed ANOVAs that expressed significant differences with Tukey–Kramer multiple comparison tests to identify which samples were significantly different. We used a Levene test to check for significant pairwise differences in sample variances, and when found, we used relative size of standard deviations to identify the samples with significantly greater variation.

## *ASPIDOSCELIS PRISCILLAE* NEW SPECIES

### Priscilla's Whiptail Lizard

Figure 1

*Holotype*. MCZ R-194296 (= SIMR 11089), a cloned adult female that also cloned herself at the SIMR (see Comments, below).

*Paratypes*. See Appendix 1, Specimens Examined. Each individual of *A. priscillae* with a MCZ or AMNH catalog number,

Figure 1. *Aspidoscelis* of New Mexico stock. Left: Adult $F_1$ female hybrid of *A. uniparens* × *A. inornatus*, AMNH R-178207. Remaining lizards, all females, left to right, represent three derived generations of *A. priscillae*, respectively as follows: $P_1$, MCZ R-193489; $F_1$, AMNH R-178211; and $F_2$, AMNH R-178213. As these individuals represent increasingly new generations from left to right, the rightmost lizard is the smallest because it is the youngest. Scale line represents 1 cm.

other than the holotype, is a paratype. Specimens with only a SIMR catalog number are not paratypes, as these were not included in morphological analyses, they were not preserved as permanent specimens, or they remain at the SIMR for now.

*Diagnosis.* A species of the *Aspidoscelis sexlineatus* species group as reviewed by Lowe et al. (1970) and Reeder et al. (2002). The species is distinguished from all others in the genus by the following combination of characters: mesoptychials moderately enlarged with mostly rounded corners; somewhat enlarged postantebrachials with corners rounded or angular; two frontoparietals; usually three parietals; four supraoculars each side; unisexual (only females exist), with reproduction by parthenogenetic cloning; body with six conspicuous longitudinal yellow or cream stripes on dark brown, gray, or black ground color (some have a partial or complete seventh vertebral light stripe); body without spots; basically no ontogenetic change in color pattern except brightness of colors and development of blue ventrally on large adults; maximum snout–vent length (SVL) about 75 mm; tetraploid number of chromosomes is 92, with four haploid sets of the *sexlineatus* species group ($n = 23$), including the four largest chromosomes being metacentric.

*Description of Holotype.* Paired data presented in the form x–y are for scale counts or femoral pores on the left–right sides of the body. SVL 66 mm; rostral large, visible from above, about as wide as high; nostril low, about central (anterior to posterior) in nasal; nasals with a long median suture behind the rostral; frontonasal hexagonal; pair of irregularly hexagonal prefrontals with a long median suture; frontal irregularly octagonal, longer than wide, wider anteriorly than posteriorly; pair of irregularly pentagonal frontoparietals with a long median suture; three irregular-sized and irregular-shaped parietals in a transverse series; irregular-sized and -shaped occipitals, posterior to but in contact with parietals, much smaller than parietals, much larger than dorsal granules. Scales in contact with outer perimeter of parietal and interparietal scales (PSC; Appendix 2) 17; back of head covered with small granules, smaller than mid-dorsal ones on body; supraoculars four, first and fourth smallest, second or third largest, separated from superciliaries by one or two rows of granules (LSG, 13-11; Appendix 2), except first supraocular broadly contacts first two superciliaries. Last supraocular separated from frontoparietals by row of small scales (circumorbital semicircles, 7-6); postnasal one on each side; loreal one on each side, large, irregular in shape; preocular one on each side, with distinct ridge (keel). A row of three suboculars, the two anterior ones with a suborbital ridge continuing anteriorly

onto the preocular, the second subocular longest. Postoculars irregular and varied in size and shape; superciliaries 6-7, the third (left) or second and third (right) longest, the posterior ones basically quadrangular. A few somewhat enlarged supratemporals; somewhat enlarged scales anterior to ear opening; central region of temple with small, roundish granules. Ear opening large, surrounded by small scales forming a smooth edge; external auditory meatus short, tympanum clearly visible; large supralabials 6-6, followed by small ones; sixth below center of eye; the third, fourth, and fifth longest. Lower eyelid with semitransparent disc of five enlarged palpebrals; pupil shape basically round to somewhat oval horizontally, with small irregularity on lower edge.

Mental trapezoid with convex anterior edge; postmental basically pentagonal; seven pairs of chin shields (=sublabials of some authors) curving posteriorly and dorsally to the lower labials, only those of anterior pair in contact at midline; chin shields, from second pair posteriorly separated in part or completely from infralabials by interlabial scales (6-9); six enlarged anterior infralabials, followed by small scales; fifth infralabial (left side) or third–sixth (right side) largest; sixth infralabial below center of eye.

Gulars small, flat, rounded, juxtaposed to slightly imbricate, somewhat larger on the anterior part of the throat, smaller posteriorly, from level of ear opening (GUL, 16; Appendix 2). Mesoptychial scales (on anterior edge of distinct, posterior, gular fold) abruptly enlarged, slightly imbricate, smooth, about 13 enlarged ones across throat. Scales on nape and side of neck similar to dorsal and lateral body scales but smaller.

Dorsal and lateral scales irregularly granular, in indistinct transverse and oblique rows; ventrals large, usually somewhat rhomboidal, usually wider than long, imbricate, smooth, mostly in eight longitudinal and 27 transverse rows (axilla to groin). Number of dorsal granules around midbody 64; preanal area with three clearly enlarged (one anterior, two posterior at cloacal opening), smooth, irregular-shaped, juxtaposed or slightly imbricate scales plus smaller ones (PAS, type I; Appendix 2).

Femoral pores 17-17, usually each pore surrounded by three small scales (medial one largest); midventrally, two scales separate the femoral pore series of each side.

Scales on dorsal and lateral aspects of tail basically irregularly rectangular, obliquely keeled, slightly mucronate, imbricate, in transverse rows, keels forming longitudinal ridges (TBS, 14; Appendix 2); scales under tail wider, smooth, more imbricate; tail round in cross-section.

Scales on upper and anterior surfaces of upper arm, on upper and anterior surfaces of forearm, on anterior and lower surfaces of thighs, and on lower surfaces of lower legs large, smooth, imbricate. Scales on lower and posterior surfaces of upper arm, on posterior and ventral surfaces of forearm, on posterior and upper surfaces of thighs, and on upper, anterior, and posterior surfaces of lower legs small, granular, juxtaposed (but postantebrachials on forearms enlarged, angular or rounded, and irregular in shape). Lamellae on ventral surface of fourth finger 16-16. Lamellae on ventral surface of fourth toe 32-32, usually in single row on fingers but often paired on toes, especially near base; fingers and toes somewhat laterally compressed; palms and soles with small, irregular, juxtaposed, flat scales (some on feet tubercular); upper surfaces of hands with large, imbricate, smooth, flat scales; anterior area of feet similar, but posterior area with granules.

*Color and Pattern of Holotype in Preservative (70% Ethanol).* Ground color of the dorsum is very dark brown. There are six complete light stripes, the lowermost two on each side (lateral and dorsolateral) being cream and basically straight, the uppermost (paravertebral) very pale yellow and slightly wavy. A short pale-yellow vertebral stripe occurs from the back of the head to

posterior to the arms, gradually fading away well anterior to midbody. There are no spots on the body. Arms above are tan to brown; lower arms with light tan lateral stripe. Legs above are brown with tan markings. Top of head is grayish brown. Base of tail, dorsally, is as the posterior body, shortly becoming unstriped light grayish blue. Ventral surfaces of the throat, chest, abdomen, tail, arms, and legs are tan.

*Colors and Pattern in Life and Ontogenetic Development*. Adults of *A. priscillae* of NM stock have a very dark brown dorsal ground color, almost black on some individuals, with six complete light stripes (Fig. 1). The lowermost (lateral) stripe is cream, the others are yellow. The light stripes are mostly straight edged, but the paravertebral light stripes usually have a slight waviness. An incomplete pale-yellow vertebral stripe on the neck usually fades away approximately above the arms. The vertebral brown area (between the paravertebral light stripes) is lighter brown than are the other dark brown fields. The dorsal aspect of the arms and legs is brown with a few tan spots or irregular stripes; often there is a short pale tan stripe, perhaps subtle, on the outer surface of the lower arm. The dorsal aspect of the hands and feet is gray. The top of the head is brown or dark olive-brown. The dorsal aspect of the tail at the base is similar to the posterior body but soon becomes pastel blue, which may be very subtle. The ventral surfaces are cream to pale blue with darker blue beneath the tail and cream beneath the arms and legs. See Appendix 1 for a list of specimens examined.

There is little ontogenetic change. Hatchlings and juveniles are similar to the adults, but have the top of the head and vertebral area of the dorsum lighter brown, the tail brighter pastel greenish blue. With age, a gray wash develops over the arms and legs. $F_1$ hybrids of *A. uniparens* × *A. inornatus* of NM stock are similar to the *A. priscillae* described above, but adult male hybrids have much more blue on them (Fig. 2).

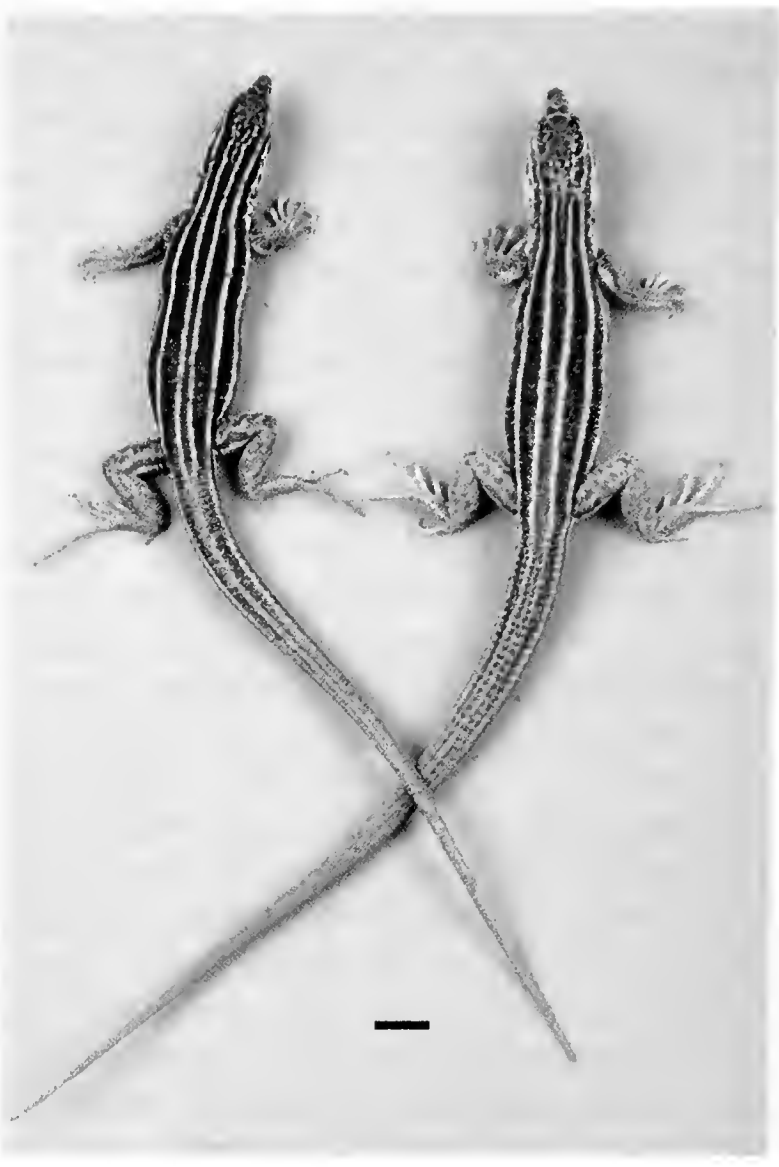


Figure 2. *Aspidoscelis* of New Mexico stock. Adult $F_1$ female hybrid (left) and male hybrid (right) of *A. uniparens* × *A. inornatus*. The female is MCZ R-193460 and the male is MCZ R-193462. Scale line represents 1 cm.

Adults of *A. priscillae* of AZ stock are similar to those of NM stock, the most conspicuous differences being that those of AZ stock usually have a complete vertebral light stripe (may be subtle or with short breaks), and the tail with more of a bluish green hue. Individuals with the most conspicuous blue on the venter may have it on the dorsal hands and feet also. The little ontogenetic change is similar to what occurs in the NM stock of *A. priscillae*, the biggest difference being that in juveniles of AZ stock, the distal tail is bluish green. $F_1$ hybrids of *A. uniparens* × *A. inornatus* of AZ stock are similar to the *A. priscillae* of AZ stock.

*Karyotype, msDNA, DNA Quantification, Reproduction, and Chromosome Pairing in*

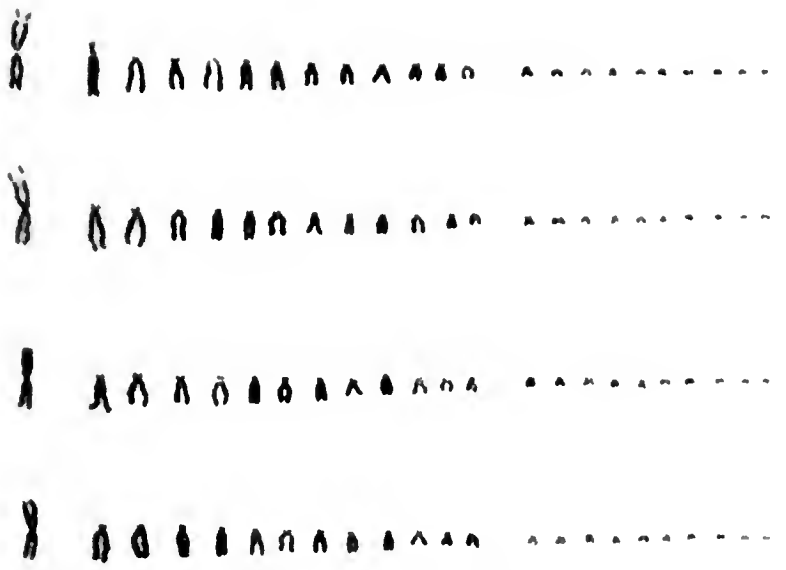
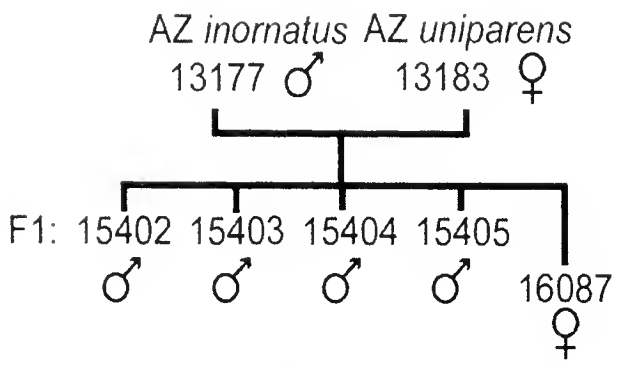


Figure 3. Tetraploid karyotype with 92 chromosomes from an embryo produced by an $F_1$ female hybrid of *A. uniparens* × *A. inornatus*. The offspring, if not sacrificed for karyotyping, would have represented the $P_1$ generation of *A. priscillae*. The egg was laid by one of the following $F_1$ hybrid females: MCZ R-193458, MCZ R-193472, AMNH R-178206, AMNH R-178207, or AMNH R-177276 (it is uncertain which one), all of which did produce offspring representing *A. priscillae*. Microchromosomes are about 1 μm in length.

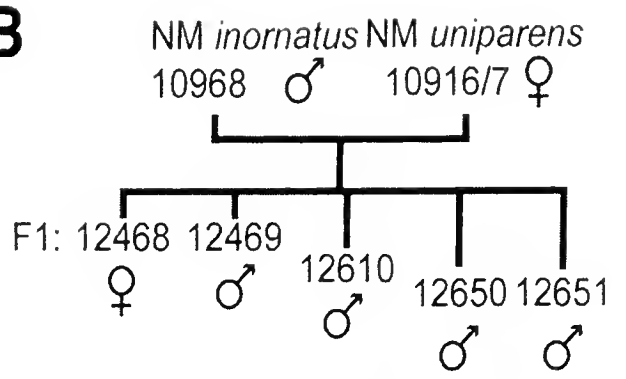
*Meiosis*. The tetraploid karyotype of *A. priscillae* (4n = 92; Fig. 3) has four haploid complements of chromosomes (n = 23) that are characteristic of the *A. sexlineatus* species group (Lowe et al., 1970; see Appendix 1 for specimens karyotyped). This haploid set consists of one set I large metacentric macrochromosome, with a secondary constriction (nucleolar organizer region [NOR], which may or may not be apparent; Ward and Cole, 1986) on one arm followed by an elongate satellite, + 12 smaller set II intermediate-sized telocentric or subtelocentric macrochromosomes (the largest is subtelocentric) + 10 set III microchromosomes (a karyotype abbreviated as n = 23 with 1 + 12 + 10 chromosomes). *Aspidoscelis inornatus* is a diploid bisexual (gonochoristic) species with two of these complements (Lowe et al., 1970) and *A. uniparens* is a triploid unisexual parthenogenetic species with three of them (Reeder et al., 2002). Given the three steps of hybridization in the evolutionary history of *A. priscillae*, which resulted in its having 4n = 92 with 4 + 48 + 40 chromosomes, three of its four haploid chromosome complements ultimately were inherited from *A. inornatus* (see below, "Evolutionary History of *A. priscillae*, Including Multiple Clones"). Usually, the NORs were apparent on two of the set I macrochromosomes in the tetraploid cells we examined for this report.

To directly examine and illustrate the parentage and genetic fingerprint of first-generation hybrids we used a panel of eight highly polymorphic microsatellite markers on two groups of animals. Each group included five first-generation hybrids and their parents derived from either AZ stock (Fig. 4A) or NM stock (Fig. 4B). The AZ *A. inornatus* father was heterozygous for all eight microsatellites, whereas the AZ *A. uniparens* mother had three distinct alleles for seven markers, consistent with the high degree of heterozygosity in this triploid unisexual species (Fig. 4C). For one microsatellite (Ai5013) two alleles were observed in *A. uniparens*, likely reflecting the presence of the same allele on two of the three homeologous chromosomes. All alleles observed in *A. uniparens* were also detected in the hybrid offspring, unequivocally identifying the maternal ancestor of the hybrids. Each hybrid offspring inherited one of the two alleles found in the AZ *A. inornatus* father. Consequently, with exceptions for Ai5013 and Cvanμ24, each microsatellite marker displayed four distinct alleles in all hybrid offspring, further confirming their parentage and tetraploid nature. Similar results were obtained for a group of first-generation hybrids and their parents of NM ancestry. In this case, homozygosity at three of the eight microsatellites for the NM *A. inornatus* father resulted in lower allele diversity among the offspring (Fig. 4D). In each group, four of the hybrids were phenotypically male and one was female. The bias toward males was less pronounced when larger numbers of first-generation hybrids of either NM or AZ stock were examined. For NM hybrids, 54% were recorded as males (36% female, 10%

**A**




**B**




**C**

| | | Ai5013 | | | | Cvanu24 | | | | MS15 | | | | MS12 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AZ *ino* | 13177 | 271 | 295 | | | 208 | 215 | | | 233 | 271 | | | 234 | 238 | | |
| AZ *uni* | 13183 | 255 | 285 | | | 202 | 208 | 257 | | 120 | 229 | 290 | | 183 | 187 | 221 | |
| AZ *hyb* | 15402 | 255 | 271 | 285 | | 202 | 208 | 257 | | 120 | 229 | 233 | 290 | 183 | 187 | 221 | 234 |
| AZ *hyb* | 15403 | 255 | 285 | 295 | | 202 | 208 | 215 | 257 | 120 | 229 | 271 | 290 | 183 | 187 | 221 | 238 |
| AZ *hyb* | 15404 | 255 | 271 | 285 | | 202 | 208 | 215 | 257 | 120 | 229 | 271 | 290 | 183 | 187 | 221 | 238 |
| AZ *hyb* | 15405 | 255 | 285 | 295 | | 202 | 208 | 215 | 257 | 120 | 229 | 233 | 290 | 183 | 187 | 221 | 234 |
| AZ *hyb* | 16087 | 255 | 285 | 295 | | 202 | 208 | 215 | 257 | 120 | 229 | 271 | 290 | 183 | 187 | 221 | 234 |

| | | MS14 | | | | MS1 | | | | MS7 | | | | MS8 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AZ *ino* | 13177 | 287 | 292 | | | 319 | 326 | | | 218 | 230 | | | 114 | 120 | | |
| AZ *uni* | 13183 | 264 | 288 | 330 | | 289 | 310 | 329 | | 239 | 254 | 289 | | 116 | 128 | 131 | |
| AZ *hyb* | 15402 | 264 | 288 | 292 | 330 | 289 | 310 | 326 | 329 | 230 | 239 | 254 | 289 | 116 | 120 | 128 | 131 |
| AZ *hyb* | 15403 | 264 | 287 | 288 | 330 | 289 | 310 | 319 | 329 | 218 | 239 | 254 | 289 | 114 | 116 | 128 | 131 |
| AZ *hyb* | 15404 | 264 | 287 | 288 | 330 | 289 | 310 | 319 | 329 | 218 | 239 | 254 | 289 | 116 | 120 | 128 | 131 |
| AZ *hyb* | 15405 | 264 | 288 | 292 | 330 | 289 | 310 | 326 | 329 | 218 | 239 | 254 | 289 | 114 | 116 | 128 | 131 |
| AZ *hyb* | 16087 | 264 | 287 | 288 | 330 | 289 | 310 | 319 | 329 | 218 | 239 | 254 | 289 | 116 | 120 | 128 | 131 |

**D**

| | | Ai5013 | | | | Cvanu24 | | | | MS15 | | | | MS12 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NM *ino* | 10968 | 237 | 299 | | | 212 | | | | 296 | | | | 143 | 249 | | |
| NM *uni* | 10916/7 | 271 | 286 | | | 202 | 206 | 265* | | 116 | 258 | 290 | | 191 | 196 | 248* | |
| NM *hyb* | 12468 | 271 | 285 | 299 | | 202 | 206 | 212 | 265 | 116 | 258 | 290 | 296 | 191 | 196 | 248* | 249 |
| NM *hyb* | 12469 | 271 | 285 | 299 | | 202 | 206 | 212 | 265 | 116 | 258 | 290 | 296 | 191 | 196 | 248* | 249 |
| NM *hyb* | 12610 | 271 | 285 | 299 | | 202 | 206 | 212 | 265 | 116 | 258 | 290 | 296 | 191 | 196 | 248* | 249 |
| NM *hyb* | 12650 | 271 | 285 | 299 | | 202 | 206 | 212 | 265 | 116 | 258 | 290 | 296 | 143 | 191 | 196 | 248* |
| NM *hyb* | 12651 | 237 | 271 | 285 | | 202 | 206 | 212 | 265 | 116 | 258 | 290 | 296 | 143 | 191 | 196 | 248* |

| | | MS14 | | | | MS1 | | | | MS7 | | | | MS8 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NM *ino* | 10968 | 312 | 344 | | | 344 | 352 | | | 245 | 270 | | | 114 | | | |
| NM *uni* | 10916/7 | 264 | 305 | 362 | | 304 | 335 | 340 | | 243 | 258 | 281 | | 116 | 121 | 131 | |
| NM *hyb* | 12468 | 264 | 304 | 344 | 362 | 304 | 335 | 340 | 344 | 243 | 245 | 258 | 281 | 114 | 116 | 121 | 131 |
| NM *hyb* | 12469 | 264 | 304 | 344 | 362 | 304 | 335 | 340 | 352 | 243 | 245 | 258 | 281 | 114 | 116 | 121 | 131 |
| NM *hyb* | 12610 | 264 | 304 | 312 | 362 | 304 | 335 | 340 | 352 | 243 | 245 | 258 | 281 | 114 | 116 | 121 | 131 |
| NM *hyb* | 12650 | 264 | 304 | 312 | 362 | 304 | 335 | 340 | 344 | 243 | 258 | 270 | 281 | 114 | 116 | 121 | 131 |
| NM *hyb* | 12651 | 264 | 304 | 344 | 362 | 304 | 335 | 340 | 344 | 243 | 258 | 270 | 281 | 114 | 116 | 121 | 131 |

Figure 4. Microsatellite (ms) DNA analysis confirms hybridization. A. Schematic tree representing relationships among specimens examined in C. Individuals are identified by their SIMR catalog number. AZ denotes specimens of Arizona stock. B. As in A, but for specimens of New Mexico (NM) stock, for which msDNA alleles are shown in D. *Aspidoscelis uniparens* 10916 and 10917 are siblings that share the same alleles at all eight msDNA markers. As we were unable to distinguish which of these two animals was the mother, both identification numbers are listed to indicate either/or. C. Microsatellite analysis for eight polymorphic markers used on genomic DNA from animals shown in A. Alleles unique to *A. inornatus* are shaded in blue; those unique to *A. uniparens* are shaded pink or red. The 208 allele (orange) of Cvanμ24 is shared by both species. Specimens labeled as *hyb* are $F_1$ hybrids. D. As in C, but for specimens of NM stock. Asterisks denote the following deviation from standard analysis: owing to pronounced crowns for longer alleles of Cvanμ24, the genotyping traces for NM *uniparens* are consistent with 265 and 267 alleles. As 267 is not observed in any other sample in this analysis we call the allele as 265 but note the uncertainty with an asterisk. For msDNA 12, the 248 allele value rounds technically to 249 in some instances. The allele is shown as 248* to distinguish it from the unambiguous 249 allele found in *A. inornatus*. For those hybrids where 248* and 249 are listed, two distinct adjacent peaks were identified.

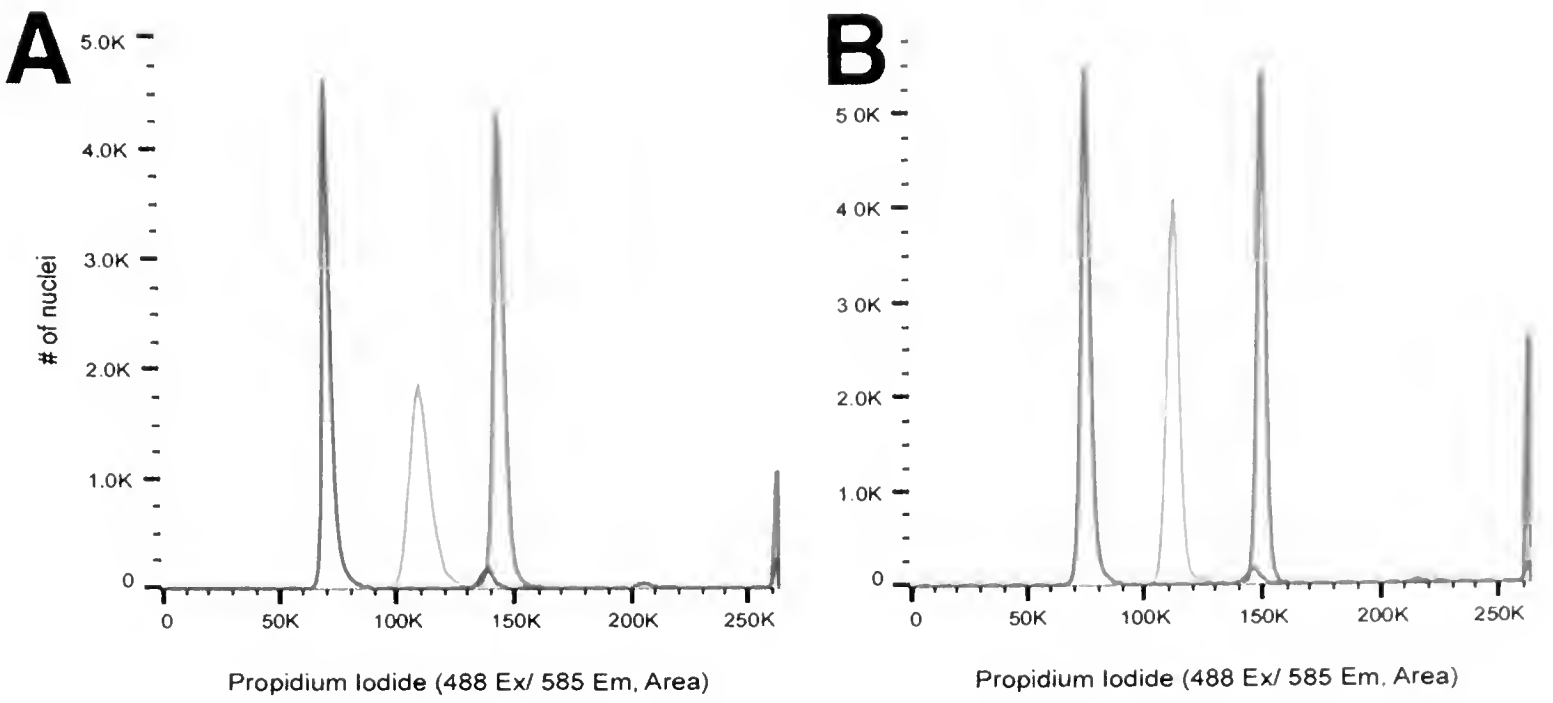



Figure 5. Clonal reproduction maintains ploidy in blood cells. Determination of DNA content by fluorescence-assisted cell sorting (FACS) of propidium iodide-stained whole blood nuclei. A. Side-by-side comparison with Arizona (AZ) *Aspidoscelis inornatus* (SIMR 15671; green) and New Mexico (NM) *A. uniparens* (SIMR 22444; blue) confirms tetraploidy for the $F_1$ hybrid of AZ stock (SIMR 15405; orange). B. As in A, but for three NM specimens: *A. inornatus* (SIMR 17247; green), *A. uniparens* (SIMR 22333; blue), and an $F_1$ hybrid (SIMR 12451; red). C. Maintenance of tetraploidy in successive generations of *A. priscillae* of AZ stock. Top: *A. uniparens* × *A. inornatus* $F_1$ hybrid (SIMR 15405); middle: *A. priscillae* $P_1$ (SIMR 18964); bottom: *A. priscillae* $F_1$ (SIMR 21081). D. Long-term maintenance of tetraploidy in *A. priscillae* of NM stock. Top, $F_1$ hybrid (SIMR 12451); middle, $F_4$ lizard (SIMR 15954); bottom, $F_6$ lizard (SIMR 20906).

undetermined as they were not examined closely for sex, $N = 56$) and for AZ hybrids 55% were males (44% female, 1% undetermined as it was not examined closely for sex, $N = 71$).

The somatic ploidy of the hybrid animals was further confirmed by FACS. Quantification of DNA content in nucleated erythrocytes showed that cells from hybrids contained twice as much DNA (within less than 0.5%) as those from AZ *A. inornatus*, consistent with a tetraploid chromosome content (Fig. 5A). As expected for a triploid species, whole blood from *A. uniparens* gave peaks centered between the other two forms (153% of *A. inornatus*, 77% of *A. priscillae*). Similar results were obtained for specimens of NM stock (Fig. 5B). As first-generation female hybrids readily reproduced in the absence of males, we surmised that the

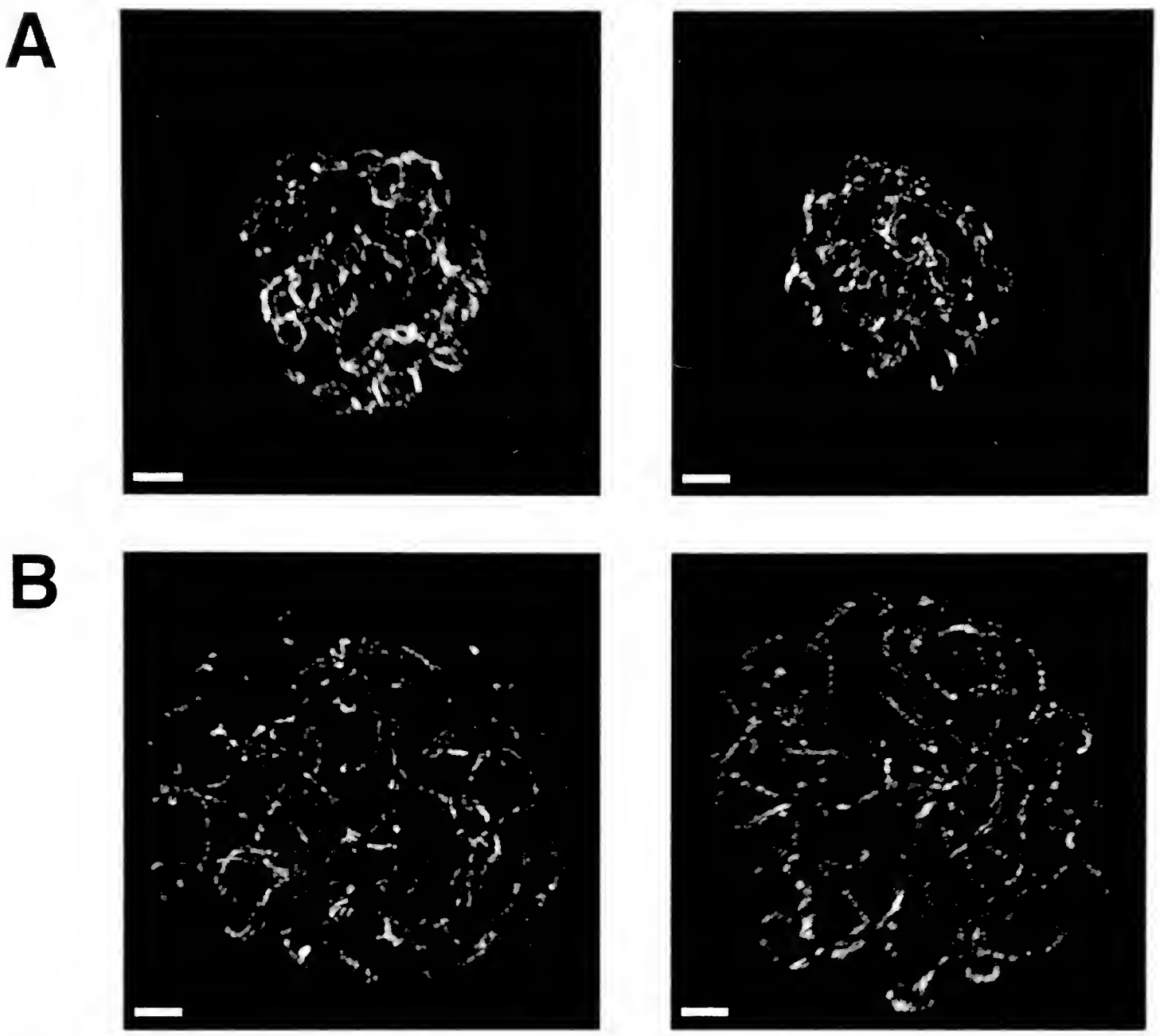



Figure 6. A. Germinal vesicles (GVs) of the diploid *Aspidoscelis tesselatus* at the diplotene stage of meiosis stained with 4′,6-diamidino-2-phenylindole and imaged by two-photon confocal microscopy. Three-dimensional projections are shown for two representative GVs (scale bar, 10 μm). B. Two representative GVs from a fourth-generation *A. priscillae* (SIMR 14442), treated and imaged as in A, indicating twice as many chromosomes.

parthenogenetic mode of reproduction was preserved from the maternal ancestor and gave rise to new clonal lineages. Indeed, DNA content analysis of AZ *A. uniparens* × *A. inornatus* hybrids and first- and second-generation *A. priscillae* revealed no significant changes in nuclear DNA content across generations (Fig. 5C). Similarly, the tetraploid DNA content did not vary among samples from the $F_1$ hybrid to the $F_6$ generation of *A. priscillae* of NM stock (Fig. 5D).

The maintenance of the tetraploid DNA content over successive generations of unisexual reproduction indicates that the meiotic mechanism previously described for other parthenogenetic species of *Aspidoscelis*, including the maternal ancestor of *A. priscillae*, was preserved. At the diplotene stage of meiosis, oocyte nuclei of *Aspidoscelis tesselatus* contain an 8C DNA content, twice the amount of DNA found in somatic cells in G2 for this species (Lutes et al., 2010). If the doubling in DNA content before the meiotic divisions failed to take place in tetraploid *A. priscillae*, diplotene oocytes would contain the same amount of chromosomal DNA as an oocyte from diploid *A. tesselatus*. In contrast, if meiosis involves transient increase in ploidy, a germinal vesicle (GV) of *A. priscillae* would contain twice as much DNA as observed in *A. tesselatus*, a prediction that is consistent with experimental results using GVs from *A.*

TABLE 2. MAJOR DIFFERENCES IN COLORS AND PATTERNS OF *ASPIDOSCELIS PRISCILLAE* AND ITS PARENTAL TAXA.

| Taxon | Vertebral Stripe | Dorsal Ground Color | Distal Tail Color | Gray or Tan Wash on Arms and Legs |
|---|---|---|---|---|
| New Mexico (NM) *A. uniparens* | usually about to arms only | brown | subtle greenish blue | more apparent |
| Arizona (AZ) *A. uniparens* | usually complete | brown | gray | more apparent |
| NM *A. inornatus* | usually complete | brown | pastel blue | more apparent |
| AZ *A. inornatus* | usually complete | lighter gray or brown | pastel blue | more apparent |
| NM *A. priscillae* | usually about to arms only | dark brown | pastel blue | less apparent |
| AZ *A. priscillae* | usually complete | dark brown | bluish green | less apparent |

*tesselatus* (Fig. 6A; Lutes et al., 2010) and a fourth-generation *A. priscillae* (Fig. 6B).

*Etymology*. The specific epithet, a noun in the genitive singular case, honors Priscilla W. Neaves, who participated equally with WBN in the capture of all whiptail lizards in the 1960s that contributed early insights into the molecular genetics, origins, and speciation of parthenogens through hybridization (Neaves and Gerald, 1968, 1969; Neaves, 1969, 1971). During the first decade of the 2000s, she also participated in capturing *A. inornatus* and *A. uniparens* in New Mexico for the laboratory hybridization project that produced most of the tetraploid lineages described here.

*Comments*. The holotype, which hatched on 13 June 2011, is a clone of an $F_1$ hybrid female, so the holotype represents the $P_1$ generation of *A. priscillae*, as she produced $F_1$-generation female offspring of *A. priscillae* (e.g., AMNH R-178226 [=SIMR 12454]). The single parent of the holotype was an $F_1$ hybrid female of *A. uniparens* × *A. inornatus* of NM stock (MCZ R-193457 [=SIMR 8975]). The parents of the $F_1$ hybrid were female *A. uniparens* (one of MCZ -194284 [=SIMR 7212], SIMR 5643, or SIMR 7803) and a male *A. inornatus* (MCZ R-192214 [=SIMR 6636]), but technically the $F_1$ hybrids do not represent a species.

Specimens that we refer to as representing *A. priscillae*, whether of NM or AZ stock, are members of multiple lineages cloned from eggs laid by multiple $F_1$ hybrid females. At least 44 $F_1$ hybrid females of *A. uniparens* × *A. inornatus* produced offspring. Some authors who strictly follow a lineage concept of species have argued that for parthenogens, each lineage arising from an $F_1$ hybrid egg constitutes a separate species, and where species boundaries are unclear, the named entity should be considered as a species complex (Frost and Wright, 1988; Frost and Hillis, 1990). We have found no reason to apply more than one specific epithet to the clones of *A. priscillae*. For additional discussion, see below ("Evolutionary History of *A. priscillae*, Including Multiple Clones").

## INTRASPECIFIC COMPARISONS

*Colors and Pattern*. Individuals of *A. priscillae* of NM and AZ stock are similar to each other in colors and pattern (see above). They differ primarily in extent of the vertebral light stripe, but this varies widely on specimens of NM stock (see Appendix 3). They also have a different tone of blue on the tail (Table 2; see above). Such differences are best appreciated by comparing side-by-side living individuals of both NM and AZ stocks. It seems possible also that the apparent differences could become blurred if we had a larger sample of AZ stock.

*Scalation*. Interspecific comparisons are discussed in detail below, but here it is appropriate to mention that no significant differences were found between the sam-

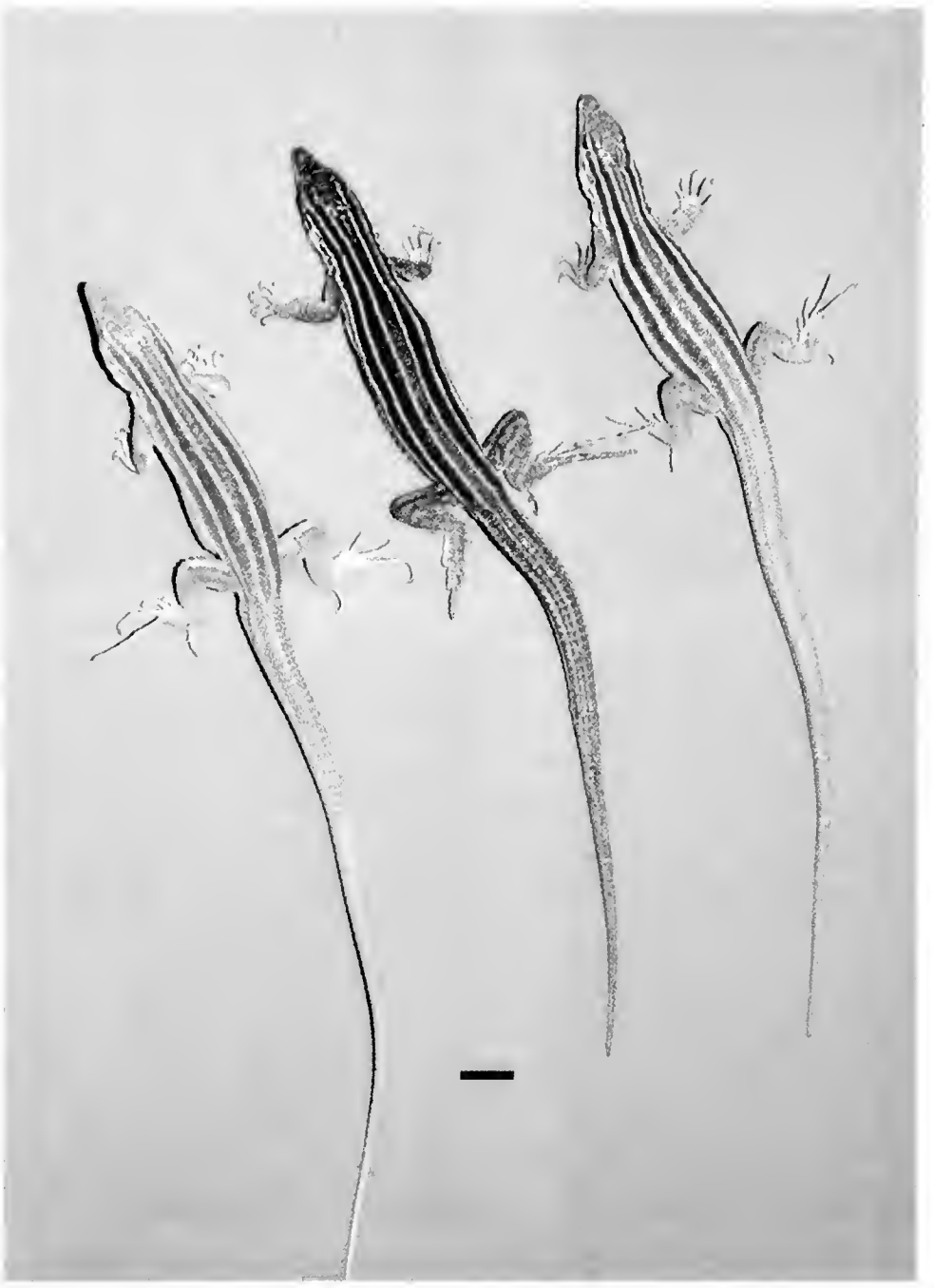


Figure 7. *Aspidoscelis* of New Mexico stock. Left: Adult male *A. inornatus*, AMNH R-178164. Right: Adult female *A. uniparens*, AMNH R-177800. Middle: Adult $F_1$ female hybrid of *A. uniparens* × *A. inornatus*, AMNH R-178207. Scale line represents 1 cm.

ples of *A. priscillae* from the NM and AZ stocks.

## INTERSPECIFIC COMPARISONS

*Colors and Pattern*. All of the taxa compared here, *A. priscillae*, *A. uniparens*, and *A. inornatus*, are similar in that they lack dorsal spots and their bodies have light cream or yellow stripes separating dark fields on the dorsum. This is because the species are all related. The triploid *A. uniparens* ultimately inherited two genomes from the diploid *A. inornatus* in its ancestry, and the tetraploid *A. priscillae* inherited a third genome of *A. inornatus* (see below, "Evolutionary History of *A. priscillae*, Including Multiple Clones"). The most conspicuous differences among these taxa in color and pattern are summarized in Table 2 and illustrated in Figure 7.

Detailed color notes for *A. priscillae* are presented above in the description. Living adults and juveniles of *A. uniparens* from NM stock are similar to *A. priscillae* of NM stock except as follows: brown on head, body, arms, and legs is a lighter tone; dorsal aspect of distal tail is subtle greenish blue rather than pastel blue; and there is a more apparent gray or tan wash on the dorsal

aspect of the arms and legs. Living adults and hatchlings of *A. uniparens* from AZ stock are similar to those of NM stock except as follows: presence of a complete vertebral light stripe (possibly subtle or with small breaks); distal tail gray, but bluish green in hatchlings.

Adults of *A. inornatus* from NM are similar to the *A. uniparens* from NM but they have a bright pastel blue tail and the light vertebral stripe is usually complete. There are only minor changes in color tones during ontogenetic development, but adult males may develop very dark blue on ventral surfaces, sides of face, snout, head above the eyes, and dorsal hands and feet. Individuals of *A. inornatus* from AZ are similar to the above, except the dorsal dark fields on the body are usually paler brown or gray in color and on some individuals the vertebral light stripe is split or partly split into two.

*Extent of the Vertebral Light Stripe*. This normally varies both between and within the species compared here (Appendix 3). For *A. inornatus*, 90% of the sample from NM had a complete stripe (neck to tail) and 100% of the sample from AZ had a complete stripe. The majority of the *A. uniparens* from NM had the stripe fade at about the arms and none had a complete stripe; the majority of the *A. priscillae* of NM stock were similar to the NM *A. uniparens* but they were more variable and 7.1% had a complete stripe. The vast majority (95.4%) of the *A. uniparens* from AZ, in strong contrast to those from NM, had a complete stripe, and 100% of the *A. priscillae* of AZ stock had a complete vertebral stripe. The $F_1$ hybrids of the NM versus AZ stock of *A. uniparens* × *A. inornatus* were similar to their maternal parental stock (0.0% of NM hybrids with complete stripe, 91.9% of AZ hybrids with complete stripe). Considering all samples compared for the extent of the vertebral light stripe, the most variable sample was the *A. priscillae* of NM stock, which might have been influenced by the pooling of multiple clones in this sample.

There is also variation in appearance of the paravertebral light stripes, but this is much more unusual than variation in the vertebral stripe described above. For example, Neaves (1971) found an apparent *A. inornatus* at Alamogordo, NM with the paravertebral stripes partially fused on the vertebral area of the dorsum (MCZ R-100080). Such aberrations as we found during this study are described here, although they are not presently useful for taxonomic or other purposes, as they seem to appear at random and from unknown causes. In *A. inornatus* of AZ stock, the vertebral stripe is partially split into two on three individuals (MCZ R-193800, MCZ R-193801, and MCZ R-193805). In *A. uniparens* of NM stock, the paravertebral stripes are fused posteriorly on the body in one individual (AMNH R-177798). In one $F_1$ hybrid of *A. uniparens* × *A. inornatus* of NM stock the paravertebral stripes converge at about midbody, then separate again without making contact (MCZ R-193460). In three $F_1$ hybrids of *A. uniparens* × *A. inornatus* of NM stock the paravertebral stripes are fused for a short distance around midbody, then separate again; the same condition occurs in one *A. priscillae* (MCZ R-193502). In one *A. priscillae* (AMNH R-177834) the left lateral and dorsolateral light stripes irregularly fuse into one stripe anterior to midbody. In two *A. priscillae* (MCZ R-194154 and AMNH R-177832) the paravertebral stripes closely approach each other (first specimen cited) or approach and touch posterior to midbody.

*Scalation and Statistics*. Embryonic development occasionally results in individuals having such abnormally high or low counts of meristic characters that they would distort the sample statistics. Retaining such specimens in statistical analyses would mean that comparisons could be generalized with confidence only when the other samples have equivalent outliers (Tabachnick and

Fidell, 2013). We identified four multivariate outliers in our sample of *A. priscillae* derived from NM stock (MCZ R-193516, AMNH R-177209, AMNH R-177826, and MCZ R-193848) and one outlier female in our AZ sample of *A. inornatus* (AMNH R-148222), which was identified by CVA as *A. uniparens*. Consequently, we excluded these five specimens from the univariate and multivariate statistical analyses.

*Univariate Comparisons of* Aspidoscelis priscillae *with Its NM Parentals*. A character-by-character examination of NM samples of *A. priscillae* and its two parental species revealed that the two parentals (*A. uniparens* and *A. inornatus*) differed significantly from each other in 9 of the 10 meristic characters (Table 3). *Aspidoscelis priscillae* was: (1) significantly different from *A. uniparens*, *A. inornatus*, and $F_1$ hybrids in SDL-T, SDL-F, and GAB; (2) resembled only *A. uniparens* in SPV and FP (also in the nonmeristic SVL, which was similar to $F_1$ hybrids also); (3) resembled only $F_1$ hybrids in COS, GUL, TBS, and PSC; and (4) resembled only *A. inornatus* and $F_1$ hybrids in LSG. Overall, *A. priscillae* of NM stock closely resembled its maternal parent, *A. uniparens*, in only 2 of the 10 meristic characters (SPV and FP). Despite the statistical differences shown in Table 3, the ranges in observed scale counts overlap to such an extent in *A. uniparens*, *A. priscillae*, and $F_1$ hybrids that many individual lizards would be difficult to identify with certainty on the basis of these individual characters.

This univariate comparison has the largest sample sizes, but they vary from character to character because the samples include specimens with complete data for all characters plus specimens with some characters missing because of damage. The larger sample sizes for the characters may provide a good univariate comparison of the species and hybrids as the characters would be observed on samples from nature.

For univariate comparisons of the smaller samples used in multivariate analyses, with complete suites of data for all 10 characters on all specimens included, we excluded the NM $F_1$ hybrids because only two $F_1$ females had complete data (Table 4). Also, this is how one would compare field samples, which would not include any known $F_1$ hybrids. Consequently, in Table 4 we compare the NM samples of *A. priscillae*, *A. uniparens*, and *A. inornatus* having complete data for all 10 characters, and these are the specimens used in the PCAs and CVAs. The individuals used are specified in Appendix 1. In these comparisons, *A. uniparens* and *A. inornatus* differed significantly in 7 of 10 meristic characters, and *A. priscillae*: (1) resembled *A. uniparens* in SDL-T, SPV, FP, and PSC; (2) resembled *A. inornatus* in LSG and TBS; and (3) differed significantly from both progenitors in COS, SDL-F, GUL, and GAB. Overall, *A. priscillae* resembled its maternal parent, *A. uniparens*, in only 4 of the 10 meristic characters (Table 4).

*Univariate Comparisons of* Aspidoscelis priscillae *with Its AZ Parentals*. Character-by-character comparisons of AZ samples showed that *A. uniparens* and *A. inornatus* were more similar to one another than were their counterparts from NM, differing significantly in fewer (7 of 10 rather than 9 of 10) univariate meristic characters (Table 5). Also, similarities between *A. priscillae* and its AZ progenitors involved different combinations of characters than shown in NM comparisons. Excluding TBS, which did not differ significantly across the four samples, *A. priscillae* from AZ stock: (1) resembled *A. uniparens* and $F_1$ hybrids in SDL-T, SPV, FP, COS, SDL-F, and LSG; (2) resembled both *A. inornatus* and $F_1$ hybrids in GAB; and (3) differed from all other groups in GUL and PSC. Overall, *A. priscillae* resembled its maternal parent, *A. uniparens*, in 7 of the 10 characters (Table 5).

The one specimen that we examined that was an $F_1$ hybrid of an NM *A. uniparens* ×

TABLE 3. DESCRIPTIVE STATISTICS AND COMPARISONS OF MEANS OF MORPHOLOGICAL CHARACTERS AMONG PARTHENOGENETIC *ASPIDOSCELIS UNIPARENS* (3N) AND GONOCHORISTIC *A. INORNATUS* (2N) FROM NEW MEXICO STOCK, THEIR $F_1$ LABORATORY HYBRIDS (4N), AND *A. PRISCILLAE* DERIVED FROM $F_1$ INDIVIDUALS BY PARTHENOGENETIC CLONING.

| | New Mexico Groups | | | |
|---|---|---|---|---|
| Character[a] | *A. inornatus* (2n) | *A. uniparens* (3n) | $F_1$ Individuals (4n) | *A. priscillae* (4n) |
| SDL-T | | | | |
| Mean ± SE | 57.0 ± 0.56 A | 64.0 ± 0.24 B | 63.4 ± 0.35 B | 65.1 ± 0.16 C |
| Range | 51–61 | 61–68 | 59–66 | 61–72 |
| *N* | 23 | 43 | 25 | 137 |
| SPV | | | | |
| Mean ± SE | 8.3 ± 0.18 C | 5.9 ± 0.12 B | 4.8 ± 0.21 A | 5.7 ± 0.07 B |
| Range | 5–10 | 0–7 | 0–6 | 0–9 |
| *N* | 38 | 58 | 37 | 156 |
| FP | | | | |
| Mean ± SE | 30.0 ± 0.36 A | 34.1 ± 0.14 C | 33.0 ± 0.27 B | 34.4 ± 0.12 C |
| Range | 27–34 | 32–37 | 30–37 | 30–40 |
| *N* | 34 | 57 | 38 | 156 |
| COS | | | | |
| Mean ± SE | 9.6 ± 0.37 A | 11.8 ± 0.14 B | 11.8 ± 0.20 BC | 12.3 ± 0.08 C |
| Range | 7–14 | 9–15 | 10–14 | 8–15 |
| *N* | 22 | 52 | 32 | 153 |
| SDL-F | | | | |
| Mean ± SE | 29.4 ± 0.47 A | 30.5 ± 0.16 B | 30.6 ± 0.28 B | 31.9 ± 0.12 C |
| Range | 25–34 | 28–32 | 27–33 | 28–36 |
| *N* | 26 | 44 | 31 | 149 |
| GUL | | | | |
| Mean ± SE | 16.1 ± 0.25 A | 18.9 ± 0.21 C | 17.9 ± 0.23 B | 17.4 ± 0.13 B |
| Range | 13–19 | 16–22 | 16–22 | 14–22 |
| *N* | 32 | 50 | 38 | 141 |
| GAB | | | | |
| Mean ± SE | 62.5 ± 0.68 A | 68.2 ± 0.18 C | 61.6 ± 0.39 A | 64.8 ± 0.15 B |
| Range | 52–70 | 66–72 | 55–65 | 60–71 |
| *N* | 33 | 57 | 36 | 154 |
| LSG | | | | |
| Mean ± SE | 23.9 ± 1.26 A | 27.6 ± 0.38 B | 25.0 ± 0.66 A | 25.4 ± 0.23 A |
| Range | 15–39 | 20–32 | 18–31 | 17–31 |
| *N* | 24 | 48 | 26 | 146 |
| TBS | | | | |
| Mean ± SE | 18.9 ± 0.37 B | 20.1 ± 0.31 C | 17.9 ± 0.40 AB | 17.6 ± 0.15 A |
| Range | 15–23 | 15–25 | 13–23 | 13–22 |
| *N* | 36 | 58 | 38 | 156 |
| PSC | | | | |
| Mean ± SE | 17.7 ± 0.75 B | 17.2 ± 0.18 B | 16.5 ± 0.29 AB | 16.4 ± 0.11 A |
| Range | 14–25 | 14–19 | 13–22 | 12–20 |
| *N* | 21 | 53 | 31 | 148 |
| SVL | | | | |
| Mean ± SE | 60.6 ± 0.62 A | 64.7 ± 0.40 B | 63.7 ± 0.57 B | 63.6 ± 0.37 B |
| Range | 52–68 | 55–72 | 55–71 | 50–73 |
| *N* | 36 | 58 | 38 | 156 |

[a] Mean ± SE, sample size, and range limits are shown for 10 univariate characters used in statistical analyses (Appendix 2). Although not used in the multivariate analyses, snout–vent length (SVL) provides a body-size comparison among the four groups. For significant differences indicated by one-way ANOVAs, we used Tukey–Kramer multiple comparison tests to determine which means were significantly different. Groups sharing the same capital letter are not significantly different at $\alpha = 0.05$.

TABLE 4. DESCRIPTIVE STATISTICS AND COMPARISONS OF MEANS OF MORPHOLOGICAL MERISTIC CHARACTERS AMONG PARTHENOGENETIC *ASPIDOSCELIS PRISCILLAE* (4N) AND ITS PROGENITOR SPECIES, PARTHENOGENETIC *A. UNIPARENS* (3N) AND GONOCHORISTIC *A. INORNATUS* (2N), FROM NEW MEXICO STOCK.

| | New Mexico Groups | | |
|---|---|---|---|
| Character[a] | *A. inornatus* (2n), $N = 12$ | *A. uniparens* (3n), $N = 25$ | *A. priscillae* (4n), $N = 108$ |
| SDL-T | | | |
| Mean ± SE | 56.8 ± 0.76 A | 64.4 ± 0.29 B | 65.1 ± 0.19 B |
| Range | 51–61 | 62–67 | 61–72 |
| SPV | | | |
| Mean ± SE | 8.4 ± 0.31 B | 6.0 ± 0.08 A | 5.7 ± 0.07 A |
| Range | 6–10 | 5–7 | 4–7 |
| FP | | | |
| Mean ± SE | 29.7 ± 0.51 A | 33.9 ± 0.20 B | 34.4 ± 0.13 B |
| Range | 27–32 | 32–36 | 30–37 |
| COS | | | |
| Mean ± SE | 9.2 ± 0.41 A | 11.6 ± 0.17 B | 12.3 ± 0.09 C |
| Range | 7–11 | 10–14 | 10–15 |
| SDL-F | | | |
| Mean ± SE | 29.4 ± 0.45 A | 30.5 ± 0.21 A | 31.8 ± 0.14 B |
| Range | 26–32 | 28–32 | 28–36 |
| GUL | | | |
| Mean ± SE | 16.0 ± 0.48 A | 18.8 ± 0.29 C | 17.4 ± 0.15 B |
| Range | 13–19 | 17–22 | 15–22 |
| GAB | | | |
| Mean ± SE | 61.7 ± 1.31 A | 68.0 ± 0.28 C | 64.8 ± 0.17 B |
| Range | 55–70 | 66–71 | 60–69 |
| LSG | | | |
| Mean ± SE | 24.4 ± 2.22 A | 28.0 ± 0.53 B | 25.3 ± 0.26 A |
| Range | 17–39 | 20–32 | 17–31 |
| TBS | | | |
| Mean ± SE | 18.8 ± 0.65 AB | 20.0 ± 0.43 B | 17.4 ± 0.18 A |
| Range | 16–22 | 17–25 | 13–22 |
| PSC | | | |
| Mean ± SE | 18.4 ± 1.12 B | 17.2 ± 0.26 AB | 16.4 ± 0.13 A |
| Range | 14–25 | 15–19 | 13–20 |
| PC1 | | | |
| Mean ± SE | 2.72 ± 0.193 B | −0.04 ± 0.071 A | -0.29 ± 0.057 A |
| Range | 1.57 to 3.71 | −0.77 to 0.50 | -1.64 to 1.18 |
| PC2 | | | |
| Mean ± SE | 0.08 ± 0.437 B | −1.39 ± 0.117 A | 0.31 ± 0.067 B |
| Range | −2.69 to 2.21 | −2.74 to -0.51 | −1.76 to 1.34 |
| CV1 | | | |
| Mean ± SE | 6.65 ± 0.394 C | 0.22 ± 0.130 B | −0.79 ± 0.098 A |
| Range | 4.02 to 8.27 | −1.66 to 1.04 | −3.67 to 1.42 |
| CV2 | | | |
| Mean ± SE | 0.57 ± 0.490 B | −2.02 ± 0.160 A | 0.40 ± 0.091 B |
| Range | −1.61 to 2.89 | −4.32 to -0.79 | −1.80 to 2.24 |

[a] Mean ± SE and range limits are shown for 10 univariate characters (Appendix 2) and 4 multivariate characters derived from principal components (PCs) and canonical variate (CV) analyses of these three groups. For significant differences indicated by one-way ANOVAs , we used Tukey–Kramer multiple comparison tests to determine which means were significantly different. Groups sharing the same capital letter are not significantly different at $\alpha = 0.05$.

*A. inornatus* from AZ was MCZ R-194410 (=SIMR 18782). This specimen was more similar to the $F_1$ hybrids of NM stock (Table 3) than to those of AZ stock (Table 5), having the following characters: SDL-T, 63; SPV, 6; FP, 34; COS, 11; SDL-F, 28; GUL, 18; GAB, 60; LSG, 28; TBS, 17; PSC, 17; and SVL, 62. Nevertheless, this specimen

TABLE 5. DESCRIPTIVE STATISTICS AND COMPARISONS OF MEANS OF MORPHOLOGICAL CHARACTERS AMONG PARTHENOGENETIC *ASPIDOSCELIS UNIPARENS* (3N) AND GONOCHORISTIC *A. INORNATUS* (2N) FROM ARIZONA STOCK, THEIR $F_1$ HYBRIDS (4N), AND *A. PRISCILLAE* DERIVED FROM $F_1$ INDIVIDUALS BY PARTHENOGENETIC CLONING.

| | Arizona Groups | | | |
|---|---|---|---|---|
| Character[a] | *A. inornatus* (2n) | *A. uniparens* (3n) | $F_1$ Individuals (4n) | *A. priscillae* (4n) |
| SDL-T | | | | |
| Mean ± SE | 54.3 ± 0.47 A | 65.9 ± 0.28 B | 65.2 ± 0.36 B | 66.4 ± 0.60 B |
| Range | 46–60 | 63–71 | 62–70 | 65–68 |
| *N* | 41 | 41 | 25 | 5 |
| SPV | | | | |
| Mean ± SE | 10.0 ± 0.17 B | 7.4 ± 0.07 A | 6.9 ± 0.11 A | 6.8 ± 0.37 A |
| Range | 8–13 | 6–9 | 6–8 | 6–8 |
| *N* | 54 | 70 | 37 | 5 |
| FP | | | | |
| Mean ± SE | 30.6 ± 0.29 A | 36.3 ± 0.15 C | 34.9 ± 0.18 B | 35.2 ± 0.73 BC |
| Range | 26–36 | 34–41 | 33–38 | 33–37 |
| *N* | 53 | 70 | 37 | 5 |
| GAB | | | | |
| Mean ± SE | 64.7 ± 0.51 A | 71.7 ± 0.20 B | 65.0 ± 0.29 A | 66.4 ± 0.60 A |
| Range | 56–74 | 68–76 | 61–70 | 65–68 |
| *N* | 52 | 69 | 37 | 5 |
| COS | | | | |
| Mean ± SE | 9.9 ± 0.30 A | 12.5 ± 0.18 B | 12.5 ± 0.17 B | 11.6 ± 0.40 B |
| Range | 7–19 | 10–16 | 11–15 | 11–13 |
| *N* | 52 | 52 | 35 | 5 |
| GUL | | | | |
| Mean ± SE | 18.5 ± 0.25 B | 17.5 ± 0.16 B | 17.7 ± 0.19 B | 16.0 ± 0.45 A |
| Range | 14–22 | 15–22 | 16–20 | 15–17 |
| *N* | 52 | 61 | 37 | 5 |
| SDL-F | | | | |
| Mean ± SE | 28.3 ± 0.25 A | 30.8 ± 0.15 B | 31.1 ± 0.24 B | 31.8 ± 0.58 B |
| Range | 24–32 | 28–34 | 28–34 | 30–33 |
| *N* | 47 | 63 | 36 | 5 |
| TBS | | | | |
| Mean ± SE | 18.8 ± 0.30 A | 19.6 ± 0.25 A | 18.7 ± 0.34 A | 18.0 ± 0.63 A |
| Range | 14–25 | 15–27 | 15–22 | 16–20 |
| *N* | 53 | 70 | 37 | 5 |
| LSG | | | | |
| Mean ± SE | 22.3 ± 0.76 A | 28.0 ± 0.39 B | 27.7 ± 0.49 B | 26.8 ± 0.86 B |
| Range | 14–37 | 22–34 | 23–34 | 25–30 |
| *N* | 52 | 51 | 34 | 5 |
| PSC | | | | |
| Mean ± SE | 17.8 ± 0.27 B | 17.8 ± 0.21 B | 18.0 ± 0.29 B | 16.2 ± 0.73 A |
| Range | 13–21 | 14–20 | 15–22 | 14–18 |
| *N* | 51 | 50 | 36 | 5 |
| SVL | | | | |
| Mean ± SE | 62.5 ± 0.73 A | 69.7 ± 0.44 B | 67.2 ± 0.46 B | 61.2 ± 1.53 A |
| Range | 51–73 | 61–76 | 60–73 | 58–67 |
| *N* | 54 | 70 | 37 | 5 |

[a] Mean ± SE, sample size, and range limits are shown for 10 univariate characters used in statistical analyses (Appendix 2). Although not used in the multivariate analyses, snout–vent length (SVL) provides a body-size comparison among the four groups. For significant differences indicated by one-way ANOVAs, we used Newman–Keuls multiple comparison tests to determine which means were significantly different. Groups sharing the same capital letter are not significantly different at $\alpha = 0.05$.

was similar to the hybrids of AZ stock in its vertebral light stripe, which was complete to the hind legs, although very inconspicuous posterior to the arms (compare with Appendix 3).

We excluded the sample of AZ $F_1$ hybrids from multivariate analyses of AZ material to make these comparisons comparable with the analyses of NM samples discussed above. Like the NM comparisons, *A. uniparens* and *A. inornatus* of AZ stock differed significantly in 7 of 10 univariate meristic characters, but NM samples also differed in GUL (Table 4), and AZ samples differed in SDL-F (Table 6). *Aspidoscelis priscillae* (1) resembled *A. uniparens* in SDL-T, FP, SDL-F, SPV, LSG, COS, GUL, PSC, and TBS; (2) resembled *A. inornatus* in GAB; and (3) resembled both *A. uniparens* and *A. inornatus* in LSG and COS. There were no significant differences among the three species for GUL, PSC, and TBS. Overall, *A. priscillae* resembled its maternal parent, *A. uniparens*, in 9 of the 10 meristic characters (Table 6).

Next, we used PCs and CVAs to convert the incongruent combinations of univariate similarities and differences to patterns of morphological variation that were more amenable to interpretation for both the NM and AZ material.

*Multivariate Comparisons of* Aspidoscelis priscillae *with its NM Progenitors*. Appendix 4 shows the PCA and CVA models for these analyses. Statistically, *A. priscillae* resembles *A. uniparens* for PC1 but resembles *A. inornatus* for PC2 (Table 4). The two-dimensional pattern of variation consists of two discrete clusters—one comprising specimens of *A. inornatus*, and a second comprising individuals of both *A. priscillae* and *A. uniparens*, which differ in PC2 (Table 4; Fig. 8).

A CVA of PCs had a classification success of 89.0%, with a classification accuracy of 83.4% (accuracy exceeding what could be attained by randomly assigning specimens to groups). The only misclassified specimens were 16 of 108 representatives of *A. priscillae*, all classified to the a priori group of *A. uniparens*. The CVA (Fig. 9) reiterated the basic two-cluster pattern shown for PCs 1 and 2, but the *A. uniparens* and *A. priscillae* clusters were separated from each other sufficiently on CV1 to produce a statistically significant difference among all three taxa (Table 4).

*Multivariate Comparisons of* Aspidoscelis priscillae *with its AZ Progenitors*. Appendix 5 shows the PCA and CVA models for these analyses. This PCA, and the PCA of NM samples, both revealed a close morphological resemblance between *A. uniparens* and *A. priscillae* (Fig. 10). New Mexico and AZ comparisons were also similar in the resemblance of *A. priscillae* to *A. uniparens* in PC1, but for AZ samples, *A. priscillae* was significantly different from both progenitor species in PC2 (Table 6).

The CVA improved classification over chance assignment by 95.2% and had an overall classification success of 96.8% (two specimens of *A. uniparens* were misclassified to the *A. priscillae* group). In contrast to the NM samples, AZ *A. priscillae* resembled *A. uniparens* in CV1 rather than being distinctively different from both progenitors, and *A. priscillae* was significantly different from both *A. uniparens* and *A. inornatus* in CV2 (Fig. 11; Table 6).

*Can Lineages Appearing to be* A. priscillae *Originate Independently from NM and AZ Progenitors?* We found no additional outliers other than the five reported above in the "Scalation and Statistics" section of "Interspecific Comparisons." We show the PCA and CVA models for this set of comparisons, with outliers removed, in Appendix 6.

Samples of *A. uniparens* from NM and AZ differed significantly in both CV1 and CV2, and the same was true for NM and AZ samples of *A. inornatus*. Therefore, one might expect that samples of *A. priscillae* from NM and AZ stock would also differ significantly, but this was not the case

TABLE 6. DESCRIPTIVE STATISTICS AND COMPARISONS OF MEANS OF MORPHOLOGICAL MERISTIC CHARACTERS AMONG PARTHENOGENETIC *ASPIDOSCELIS PRISCILLAE* (4N) AND ITS PROGENITOR SPECIES, PARTHENOGENETIC *A. UNIPARENS* (3N) AND GONOCHORISTIC *A. INORNATUS* (2N) FROM ARIZONA STOCK.

| | Arizona Groups | | |
|---|---|---|---|
| Character[a] | *A. inornatus* (2n), $N = 38$ | *A. uniparens* (3n), $N = 20$ | *A. priscillae* (4n), $N = 5$ |
| SDL-T | | | |
| Mean ± SE | 54.3 ± 0.49 A | 66.0 ± 0.42 B | 66.4 ± 0.60 B |
| Range | 46–60 | 63–71 | 65–68 |
| FP | | | |
| Mean ± SE | 30.7 ± 0.36 A | 36.4 ± 0.28 B | 35.2 ± 0.73 B |
| Range | 26–36 | 35–39 | 33–37 |
| SDL-F | | | |
| Mean ± SE | 28.3 ± 0.26 A | 31.0 ± 0.23 B | 31.8 ± 0.58 B |
| Range | 26–32 | 29–33 | 30–33 |
| GAB | | | |
| Mean ± SE | 64.7 ± 0.61 A | 71.6 ± 0.37 B | 66.4 ± 0.60 A |
| Range | 56–74 | 69–76 | 65–68 |
| SPV | | | |
| Mean ± SE | 9.8 ± 0.22 B | 7.4 ± 0.13 A | 6.8 ± 0.37 A |
| Range | 8–13 | 6–8 | 6–8 |
| LSG | | | |
| Mean ± SE | 22.6 ± 0.93 A | 27.6 ± 0.66 B | 26.8 ± 0.36 AB |
| Range | 14–37 | 22–34 | 25–30 |
| COS | | | |
| Mean ± SE | 9.8 ± 0.38 A | 12.5 ± 0.36 B | 11.6 ± 0.40 AB |
| Range | 7–19 | 10–16 | 11–13 |
| GUL | | | |
| Mean ± SE | 18.2 ± 0.26 A | 17.8 ± 0.25 A | 16.0 ± 0.45 A |
| Range | 14–21 | 16–20 | 15–17 |
| PSC | | | |
| Mean ± SE | 17.8 ± 0.30 A | 17.6 ± 0.39 A | 16.2 ± 0.73 A |
| Range | 14–21 | 14–20 | 14–18 |
| TBS | | | |
| Mean ± SE | 18.4 ± 0.32 A | 19.3 ± 0.50 A | 18.0 ± 0.63 A |
| Range | 14–23 | 16–23 | 16–20 |
| PC1 | | | |
| Mean ± SE | -0.74 ± 0.077 A | 1.15 ± 0.053 B | 1.01 ± 0.100 B |
| Range | −1.68 to 0.50 | 0.73 to 1.65 | 0.77 to 1.29 |
| PC2 | | | |
| Mean ± SE | 0.03 ± 0.172 B | 0.22 ± 0.166 B | −1.15 ± 0.341 A |
| Range | −3.03 to 1.95 | −1.53 to 1.43 | −2.10 to −0.29 |
| CV1 | | | |
| Mean ± SE | 2.18 ± 0.199 B | −3.48 ± 0.099 A | −2.66 ± 0.202 A |
| Range | 0.08 to 4.78 | −4.18 to −2.77 | −3.07 to −1.89 |
| CV2 | | | |
| Mean ± SE | 0.03 ± 0.174 B | 0.36 ± 0.204 B | −1.66 ± 0.310 A |
| Range | −3.19 to 1.69 | −1.44 to 2.17 | −2.62 to −0.82 |

[a] Mean ± SE and range limits are shown for 10 univariate characters (Appendix 2) and 4 multivariate characters derived from principal components (PCs) and canonical variate (CV) analyses of these three groups. For significant differences indicated by one-way ANOVAs, we used Tukey–Kramer multiple comparison tests to determine which means were significantly different. Groups sharing the same capital letter are not significantly different at $\alpha = 0.05$.

(Table 7). This suggests that separate lineages appearing to represent *A. priscillae* could originate in nature independently from $F_1$ hybrids of syntopic *A. uniparens* and *A. inornatus* regardless of geographic provenance of the progenitor species.

*Additional Details.* All individuals of all taxa, population samples, and hybrids compared had eight rows of enlarged ventral

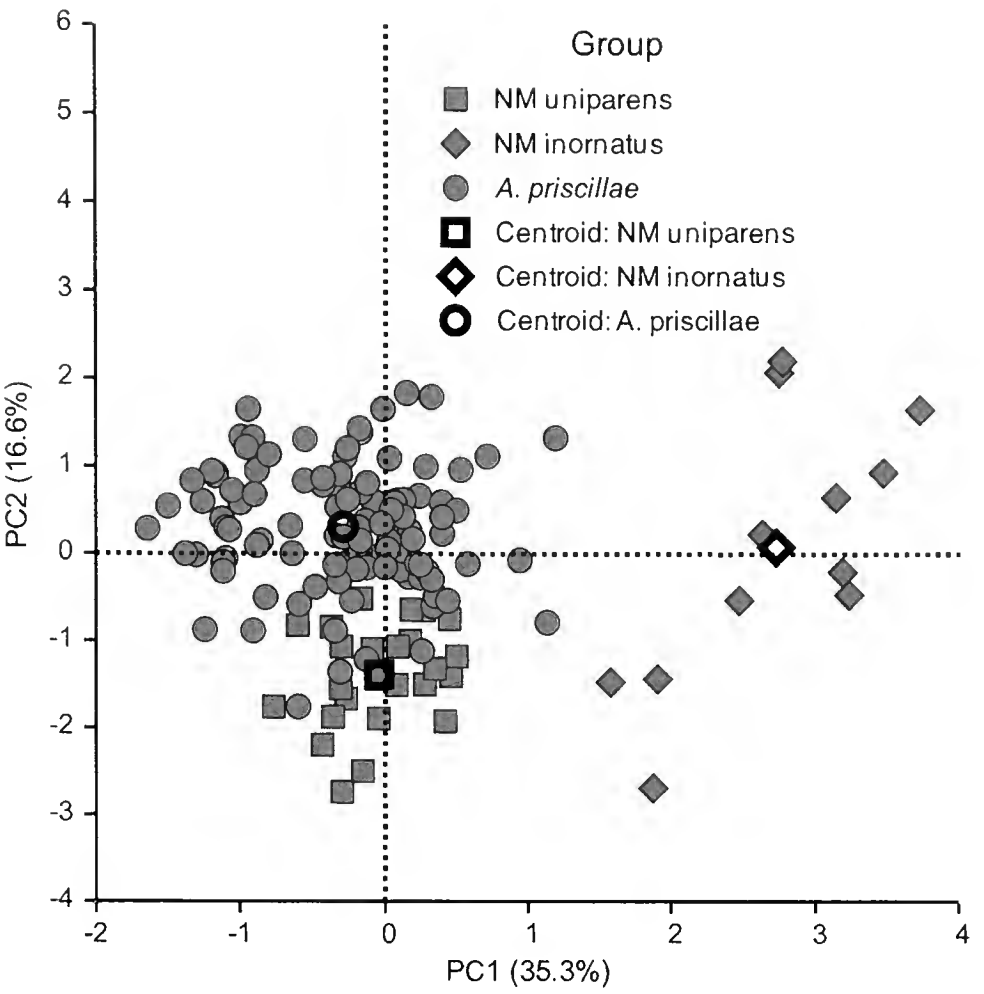



Figure 8. Scatter plot of principal component (PC) scores of the tetraploid *Aspidoscelis priscillae* and its progenitor species, *A. uniparens* and *A. inornatus* of New Mexico stock. The PCA model (Appendix 4) was based on the scale counts and samples shown in Table 4. Axis percentages are proportions of variance accounted for by PC1 and PC2.

plates across the belly at midbody. Additional characters examined are as follows.

Condition of enlarged ventral preanal scales: the character states are of three types, as specified in Appendix 2. Comparing individuals of all the taxa, population samples, and hybrids, the most common condition in each group was type I, except for $F_1$ hybrids of AZ stock (most individuals had type II) and both the NM and AZ stocks of *A. priscillae*, which mostly had type III. Nevertheless, each sample showed significant variation (Appendix 7).

Number of interfemoral scales: these are the midventral scales that separate the series of femoral pores on the opposite legs. Including all taxa, the overall range of variation for this character was one to five scales. The *A. priscillae* of both NM and AZ stock usually had two scales, similar to the *A. uniparens* of both NM and AZ stock. The majority of the *A. inornatus* of both NM and AZ stock had three scales (Appendix 8).

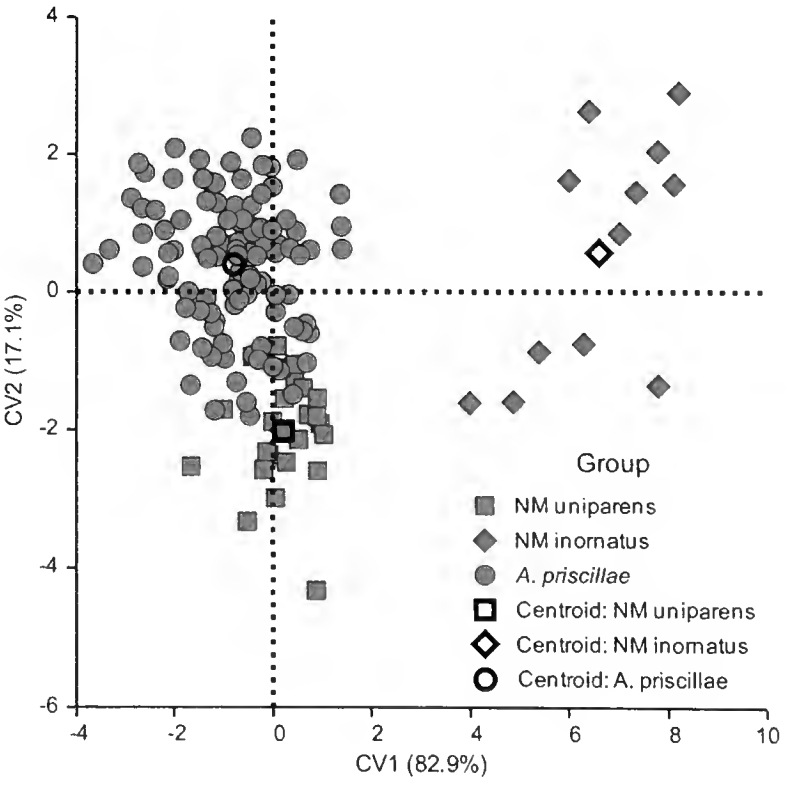



Figure 9. Scatter plot of canonical variate (CV) scores of the tetraploid *Aspidoscelis priscillae* and its progenitor species, *A. uniparens* and *A. inornatus* of New Mexico stock. The CV analysis model was based on the samples shown in Table 4 and the principal components (used as characters) shown in Appendix 4. Axis percentages are proportions of among-groups variance accounted for by CV1 and CV2.

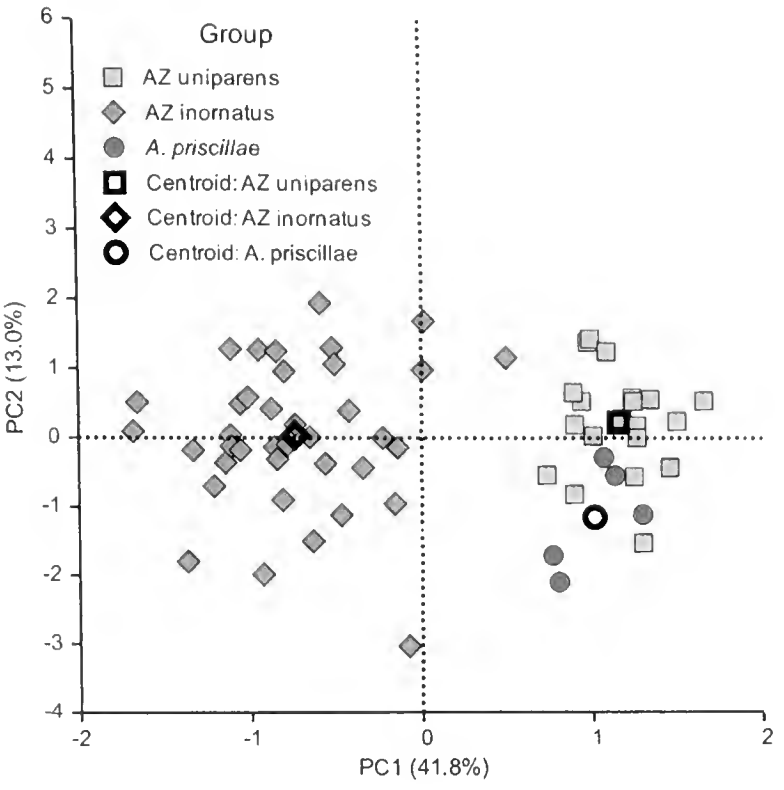



Figure 10. Scatter plot of principal component (PC) scores of the tetraploid *Aspidoscelis priscillae* and its progenitor species, *A. uniparens* and *A. inornatus* of Arizona stock. The PC analysis model (Appendix 5) was based on the univariate characters and samples shown in Table 6. Axis percentages are proportions of variance accounted for by PC1 and PC2.

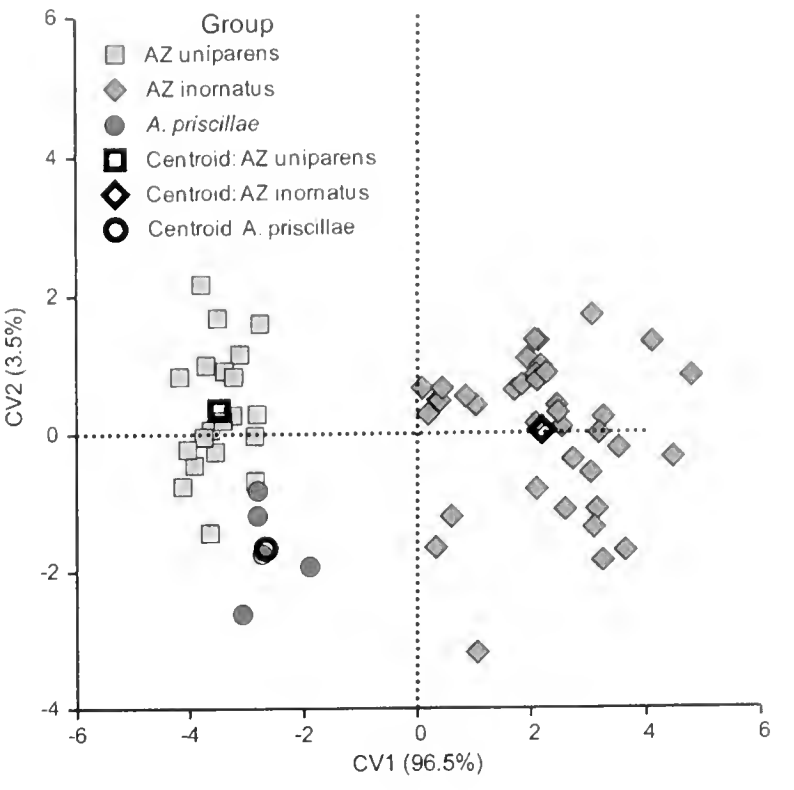



Figure 11. Scatter plot of canonical variate (CV) scores of the tetraploid *A. priscillae* and its progenitor species, *A. uniparens* and *A. inornatus* of Arizona stock. The CV analysis model was based on the samples shown in Table 6 and the principal components (used as characters) shown in Appendix 5. Axis percentages are proportions of among-groups variance accounted for by CV1 and CV2.

Condition of postantebrachial scales on the forearm: in specimens of *A. inornatus* from NM, the scales are slightly enlarged and have either rounded or angular corners. In *A. inornatus* from AZ and *A. uniparens* from both NM and AZ, these scales are similar to those of *A. inornatus* from NM but somewhat larger. In *A. priscillae* of both NM and AZ stock, these scales were a bit larger yet.

Condition of mesoptychial scales across the throat: In individuals of all samples of the taxa compared, these scales were moderately enlarged with mostly rounded corners.

## IDENTIFYING PUTATIVE HYBRIDS FROM NATURE

Four specimens, three males and one female collected from two sites in NM (see Appendix 1), were previously identified as possible hybrids of *A. uniparens* and *A. inornatus* largely on the basis of sex and a hybrid index derived from the SAB (= GAB) character (Wright, 1968). Because the putative hybrids are from NM, we used NM samples of *A. uniparens*, *A. inornatus*, and NM $F_1$ hybrids as a priori groups in a CVA (Table 8). The four putative hybrids were included in the CVA as "unknowns" for classification to the a priori group that each most closely resembled. The PCA and CVA models for these analyses are shown in Appendix 9.

Classification success was 91.7%, with two individuals of *A. uniparens* misclassified to the hybrid group and two individuals of the presumptive hybrid group misclassified to *A. uniparens*. None of the specimens of *A. inornatus* was misclassified. The model provided a classification accuracy of 87.5%; i.e., accuracy greater than what would be expected from random assignment of specimens to groups.

The CVA assigned specimens MSB 7794 and MSB 7922 (both males) to the a priori $F_1$ hybrid group with probabilities of 88.7% and 90.7%, respectively. However, speci-

TABLE 7. DESCRIPTIVE STATISTICS OF PRINCIPAL COMPONENTS (PCs) AND CANONICAL VARIATES (CVs) BETWEEN PAIRED SAMPLES OF PARTHENOGENETIC *ASPIDOSCELIS UNIPARENS* AND GONOCHORISTIC *A. INORNATUS* FROM NEW MEXICO AND ARIZONA, AND THEIR HYBRIDIZATION DERIVATIVE, PARTHENOGENETIC *A. PRISCILLAE*.

| | Multivariate Characters[b] | | | |
|---|---|---|---|---|
| Paired Groups[a] | PC1 | PC2 | CV1 | CV2 |
| New Mexico (NM) *A. uniparens* (3n) | | | | |
| Mean ± SE | 0.29 ± 0.048 | −0.92 ± 0.117 | 1.04 ± 0.128 | 1.05 ± 0.128 |
| Range | −0.14 to 0.64 | −2.33 to −0.02 | −0.30 to 2.55 | −0.24 to 2.88 |
| *N* | | 25 | | |
| Arizona (AZ) *A. uniparens* (3n) | | | | |
| Mean ± SE | 0.67 ± 0.084 | −1.44 ± 0.112 | 1.53 ± 0.129 | 2.79 ± 0.164 |
| Range | 0.11 to 1.57 | −2.26 to −0.32 | 0.62 to 2.84 | 1.68 to 3.81 |
| *N* | | 20 | | |
| Significant difference? | Yes: $P < 0.001$ | Yes: $P = 0.003$ | Yes: $P = 0.012$ | Yes: $P < 0.001$ |
| NM *A. inornatus* (2n) | | | | |
| Mean ± SE | −1.46 ± 0.159 | 0.39 ± 0.387 | −4.31 ± 0.306 | −2.10 ± 0.465 |
| Range | −2.23 to −0.48 | −2.16 to 2.46 | −6.09 to −2.91 | −4.45 to 0.815 |
| *N* | | 12 | | |
| AZ *A. inornatus* (2n) | | | | |
| Mean ± SE | −1.63 ± 0.099 | −0.23 ± 0.152 | −5.64 ± 0.252 | 0.40 ± 0.229 |
| Range | −2.78 to 0.05 | −2.40 to 2.05 | −9.60 to −2.33 | −2.63 to 2.67 |
| *N* | | 38 | | |
| Significant difference? | No: $P = 0.397$ | No: $P = 0.081$ | Yes: $P = 0.008$ | Yes: $P < 0.001$ |
| NM *A. priscillae* (4n) | | | | |
| Mean ± SE | 0.52 ± 0.038 | 0.52 ± 0.062 | 1.88 ± 0.086 | −0.67 ± 0.082 |
| Range | −0.53 to 1.41 | −1.55 to 1.80 | −0.24 to 4.08 | −2.24 to 1.39 |
| *N* | | 107 | | |
| AZ *A. priscillae* (4n) | | | | |
| Mean ± SE | 0.54 ± 0.096 | 0.11 ± 0.288 | 1.77 ± 0.173 | −0.06 ± 0.338 |
| Range | 0.27 to 0.73 | −0.51 to 0.89 | 1.44 to 2.43 | −1.29 to 0.70 |
| *N* | | 5 | | |
| Significant difference? | No: $P = 0.910$ | No: $P = 0.164$ | No: $P = 0.796$ | No: $P = 0.120$ |

[a] Independent sample *t* tests, rather than paired *t* tests, were used.

[b] Mean ± SE and range limits are shown for the first two PCs from a PC analysis of six combined samples and CVs from a CV analysis of the resulting PCs. Models for these analyses are shown in Appendix 6.

mens MSB 7795 (a female) and MSB 7796 (a male) were assigned to *A. inornatus* with probabilities of 100% and 99.9% respectively. The reliability of these CVA classifications is unmistakable in the spatial relationships of the MSB specimens to the a priori groups in two dimensions (Fig. 12).

## COMPARATIVE VARIATION IN UNISEXUAL AND BISEXUAL SAMPLES

*Relative Variability among Samples of* Aspidoscelis uniparens, A. inornatus, *and* A. priscillae *from NM Stock*. We use the term variability in a statistical sense to refer to the extent to which individuals in a sample diverge from the mean and from each other as reflected by the sample variance or standard deviation. The basic question here and in the next section is whether the unisexual clonal taxa are less variable than the bisexual taxon, as one might expect. Also, might the variability of the $F_1$ hybrids be intermediate to that of their parents?

We decided to exclude the sample of NM $F_1$ hybrids from these analyses because $F_1$ hybrid females with complete data for all 10 characters were underrepresented ($N$ = nine males, two females), and subsamples of males and females had heterogeneous variances for PC1 and PC2. The three remaining samples to be compared differed dramatically in sample size (*A. priscillae*, $N$ = 108; *A. uniparens*, $N$ = 25; and *A.*

TABLE 8. DESCRIPTIVE STATISTICS AND COMPARISONS OF MEANS OF UNIVARIATE CHARACTERS AND THE FIRST TWO PRINCIPAL COMPONENTS (PCs) AND CANONICAL VARIATES (CVs) AMONG PARTHENOGENETIC *ASPIDOSCELIS UNIPARENS* AND GONOCHORISTIC *A. INORNATUS* FROM NEW MEXICO STOCK AND THEIR $F_1$ LABORATORY HYBRIDS. FOUR SPECIMENS, IDENTIFIED PREVIOUSLY AS POSSIBLE HYBRIDS (WRIGHT, 1968), WERE INCLUDED IN THE CV ANALYSIS AS UNCLASSIFIED INDIVIDUALS FOR ASSIGNMENT TO THE GROUP THAT EACH MOST CLOSELY RESEMBLED.

| | Groups | | | Hybrids? | | | |
|---|---|---|---|---|---|---|---|
| Character[a] | *A. inornatus* (2n), $N = 12$ | *A. uniparens* (3n), $N = 25$ | $F_1$ tetraploids, $N = 11$ | MSB 7794, Male | MSB 7795, Female | MSB 7796, Male | MSB 7922, Male |
| SDL-T | | | | 63 | 55 | 59 | 59 |
| Mean ± SE | 56.8 ± 0.76 A | 64.4 ± 0.29 B | 63.7 ± 0.49 B | | | | |
| Range | 51–61 | 62–67 | 61–66 | | | | |
| SPV | | | | 6 | 7 | 6 | 5 |
| Mean ± SE | 8.4 ± 0.31 B | 6.0 ± 0.08 A | 5.5 ± 0.16 A | | | | |
| Range | 6–10 | 5–7 | 5–6 | | | | |
| FP | | | | 34 | 31 | 33 | 36 |
| Mean ± SE | 29.7 ± 0.51 A | 33.9 ± 0.20 B | 33.0 ± 0.50 B | | | | |
| Range | 27–32 | 32–36 | 30–36 | | | | |
| COS | | | | 9 | 6 | 6 | 11 |
| Mean ± SE | 9.2 ± 0.41 A | 11.6 ± 0.17 B | 11.9 ± 0.25 B | | | | |
| Range | 7–11 | 10–14 | 11–13 | | | | |
| GUL | | | | 17 | 15 | 18 | 17 |
| Mean ± SE | 16.0 ± 0.48 A | 18.8 ± 0.29 B | 17.6 ± 0.24 B | | | | |
| Range | 13–19 | 17–22 | 16–19 | | | | |
| GAB | | | | 63 | 67 | 65 | 65 |
| Mean ± SE | 61.2 ± 1.31 A | 68.0 ± 0.28 B | 62.0 ± 0.49 A | | | | |
| Range | 55–70 | 66–71 | 60–64 | | | | |
| LSG | | | | 22 | 22 | 29 | 27 |
| Mean ± SE | 24.4 ± 2.22 A | 28.0 ± 0.53 A | 26.5 ± 1.11 A | | | | |
| Range | 17–39 | 20–32 | 18–31 | | | | |
| SDL-F | | | | 32 | 29 | 29 | 31 |
| Mean ± SE | 29.4 ± 0.45 A | 30.5 ± 0.21 AB | 30.7 ± 0.43 B | | | | |
| Range | 26–32 | 28–32 | 28–33 | | | | |
| TBS | | | | 18 | 20 | 20 | 15 |
| Mean ± SE | 18.8 ± 0.65 A | 20.0 ± 0.43 A | 18.6 ± 0.53 A | | | | |
| Range | 16–22 | 17–25 | 16–21 | | | | |
| PSC | | | | 18 | 16 | 14 | 14 |
| Mean ± SE | 18.4 ± 1.12 A | 17.2 ± 0.26 A | 17.4 ± 0.53 A | | | | |
| Range | 14–25 | 15–19 | 15–22 | | | | |
| PC1 | | | | 0.14 | 1.51 | 0.52 | −0.26 |
| Mean ± SE | 1.52 ± 0.186 C | −0.69 ± 0.064 A | −0.26 ± 0.084 B | | | | |
| Range | 0.51 to 2.43 | −1.30 to −0.27 | −0.81 to 0.21 | | | | |
| PC2 | | | | 0.30 | −0.41 | −1.11 | −1.24 |
| Mean ± SE | 0.25 ± 0.487 A | −0.04 ± 0.142 A | 0.03 ± 0.185 A | | | | |
| Range | −1.76 to 2.85 | −1.87 to 1.17 | −0.71 to 1.10 | | | | |
| CV1 | | | | 0.24 | −5.50 | −2.81 | 1.10 |
| Mean ± SE | −5.11 ± 0.325 B | 1.85 ± 0.197 A | 1.38 ± 0.267 **A** | | | | |
| Range | −7.68 to −3.33 | 0.02 to 4.69 | 0.48 to 3.08 | | | | |
| CV2 | | | | 1.17 | −1.88 | −2.02 | 1.52 |
| Mean ± SE | −0.08 ± 0.428 A | −0.51 ± 0.147 A | 1.25 ± 0.267 B | | | | |
| Range | −2.08 to 2.23 | −2.22 to 0.91 | −0.37 to 3.14 | | | | |
| Group assignment by CVA | | | | | | | |
| Group | | | | $F_1$ | *A. inornatus* | *A. inornatus* | $F_1$ |
| *P* (%) | | | | 88.7 | 100 | 99.9 | 90.7 |

[a] Mean ± SE and range limits are shown for 10 univariate characters and 4 multivariate characters derived from PC and CV analyses of these three groups. We followed one-way ANOVAs with Tukey multiple comparison tests to determine which means were significantly different. Groups sharing the same capital letter are not significantly different at $\alpha = 0.05$.

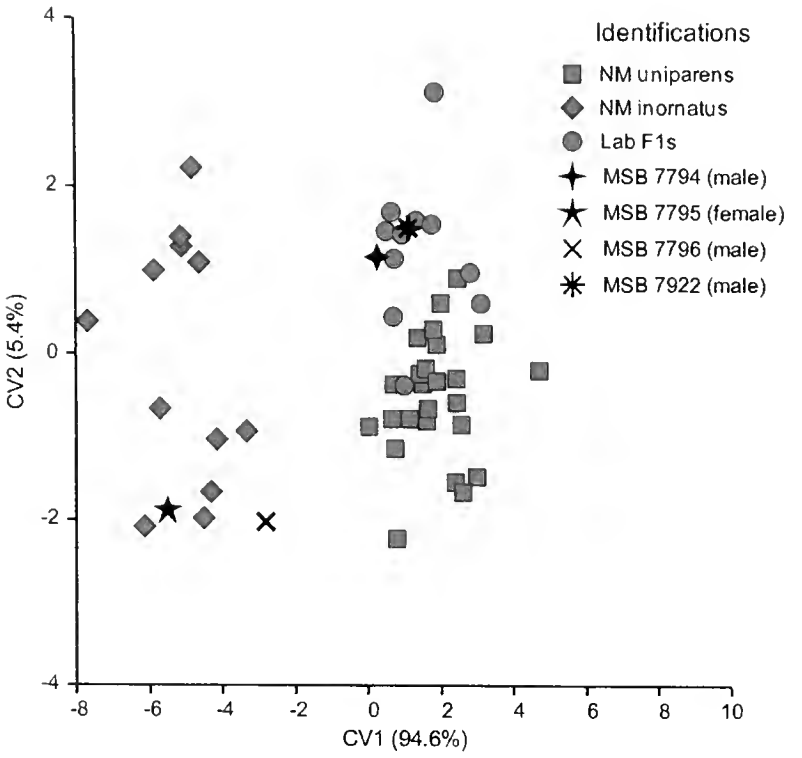



Figure 12. Scatter plot of canonical variate (CV) scores of *Aspidoscelis uniparens*, *A. inornatus*, and tetraploid $F_1$ hybrids of *A. uniparens* × *A. inornatus* of New Mexico stock. Four MSB specimens (putative hybrids collected in the field about 50 years ago) were included as unclassified individuals for assignment to the most similar a priori group. The CV analysis model was based on samples shown in Table 8 and the principal components (used as characters) shown in Appendix 9. Axis percentages are proportions of among-groups variance accounted for by CV1 and CV2.

*inornatus*, $N = 12$). Therefore, we used the RANDBETWEEN formula in Excel© to randomly select 12 specimens each from the much larger samples of *A. uniparens* and *A. priscillae* to adjust for possible differences in variability owing to sample size. The PCA model is shown in Appendix 10.

On the basis of the proportion of variation summarized, we used PC1 as the best measure of relative variability in these comparisons and standard deviations (SD) in lieu of variances to reflect similarities and differences. There were no significant differences in the sample variances for this character: *A. uniparens* and *A. inornatus* (SD = 0.27 and 0.52, respectively; $P = 0.08$); *A. uniparens* and *A. priscillae* (SD = 0.27 and 0.48, respectively; $P = 0.12$); and *A. inornatus* and *A. priscillae* (SD = 0.52 and 0.48, respectively; $P = 0.76$). This was consistent with the comparative unpredictability of meristic variability between parthenogenetic and sexually reproducing species in nature (Taylor et al., 2012) and in the laboratory (Cole et al., 2016). Also, it is possible that the similarity of all three samples compared here may reflect the multiple origins of the *A. priscillae* from many $F_1$ hybrid eggs (i.e., multiple clones).

*Relative Variability among Samples of* Aspidoscelis uniparens, A. inornatus, *and $F_1$ Hybrids from AZ Stock*. We excluded the sample of *A. priscillae* from this set of comparisons because of its small sample size ($N = 5$). However, a preliminary PCA of $F_1$ hybrids revealed no significant differences in the variances of PC1 between samples of 15 males and 9 females ($P = 0.27$), so we pooled the sexes of the $F_1$ hybrids for this analysis. We used the RANDBETWEEN formula in Excel to randomly select 19 specimens from samples of *A. uniparens* and *A. inornatus* to adjust sample sizes to that of the $F_1$ hybrids with complete data. This was to adjust for possible artifactual differences in variability due to sample size. The PCA model is shown in Appendix 10.

Without the information on variation from Taylor et al. (2012), Cole et al. (2016), and the NM tests discussed above, we might have hypothesized that samples of *A. inornatus* and $F_1$ hybrids would have exhibited greater meristic variability than *A. uniparens* because each individual originated from a fertilization involving a genetically different spermatozoon. However, the opposite was true; *A. uniparens* and *A. inornatus* had homogeneous variances for PC1 (SD = 0.29 and 0.54, respectively; $P = 0.05$), as did the samples of *A. uniparens* and $F_1$ hybrids (SD = 0.29 and 0.24, respectively; $P = 0.42$). For the two AZ groups originating from fertilizations, *A. inornatus* was more variable than the $F_1$ hybrids (SD = 0.54 and 0.24, respectively; $P = 0.02$). Therefore, consistent with previous studies, a clonal species (*A. uniparens*) may express as much morphological variation as a sexually reproducing species (*A. inornatus*).

## EVOLUTIONARY HISTORY OF *A. PRISCILLAE*, INCLUDING MULTIPLE CLONES

*Ancestry*. The ancestry of *A. priscillae* involved three steps of hybridization. Its most recent maternal parent, *A. uniparens*, had originated previously in nature through two steps of hybridization, and the third step, which produced *A. priscillae*, was in the laboratory at the SIMR, where individuals of *A. uniparens* mated with males of *A. inornatus*. As reviewed by Reeder et al. (2002), the first step of hybridization involved a female of *A. inornatus* crossed with a male of *Aspidoscelis barrancorum*, *Aspidoscelis costatus*, or *Aspidoscelis stictogrammus* (in large part depending on one's taxonomy), probably in northern Mexico. Although it is uncertain whether the resultant form survives somewhere today, it is likely to be or have been a diploid, clonal, parthenogen that backcrossed with *A. inornatus*, resulting in the triploid, parthenogenetic, clonal *A. uniparens*. As a consequence of the laboratory hybridization of *A. uniparens* × *A. inornatus*, a third genome of *A. inornatus* was added in producing the tetraploid *A. priscillae*. Consequently, *A. priscillae* inherited 100% of the three genomes of *A. uniparens* and, ultimately, this included three genomes of *A. inornatus* through separate hybridization events in its evolutionary history. Nevertheless, today *A. priscillae* most closely resembles its maternal parent (Figs. 8–11; see above).

*Multiple Clones versus Multiple Species*. In the laboratory at SIMR, at least 15 different females of *A. uniparens* mated with several different males of *A. inornatus* to produce viable hybrids, and stock from both NM and AZ were included for both parents. At least 116 $F_1$ hybrid individuals were formed, with an approximate 50:50 sex ratio. Of these, at least 44 $F_1$ hybrid females produced tetraploid clones. In the laboratory, we initially relied on using msDNA for 12 loci to identify individual lizards, hybrids, and clones, but it turned out that the similarities at these particular loci were so great in the offspring of *A. uniparens* × *A. inornatus* that in many instances individuals and clones could not be identified with confidence (see above).

The lineage theory of species has been strictly interpreted to mean that for parthenogens, the cloned lineage resulting from each $F_1$ hybrid zygote is a separate species (Frost and Wright, 1988; Frost and Hillis, 1990), although this has not been universally accepted (e.g., Cole, 1990). If we were to follow that interpretation, we would have at least 44 species to name among the specimens examined for this report, and most of them would be indistinguishable or impossible to diagnose or identify. We find that the best way to deal with this material is to provide one scientific name, as all individuals of *A. priscillae* were formed from gene pools of the same two parental species. If one refers to *A. priscillae* as a species complex that includes many lineages (Frost and Wright, 1988), this should be satisfactory to those who prefer the strict lineage concept of species as well as those who prefer other species concepts that involve processes at the population level, not the individual level (e.g., Coyne and Orr, 2004). In any event, different clones also originate from sources other than different $F_1$ hybrid females, such as postformation mutations in parthenogens, and apparently several clones of *A. uniparens* are known to exist (Dessauer and Cole, 1989). These lineages differ in certain allozymes, but we will not name them as separate species.

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Original data underlying this manuscript can be accessed from the Stowers Original Data Repository at http://www.stowers.org/research/publications/LIBPB-1211.

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## APPENDIX 1. SPECIMENS EXAMINED

The specimens examined are listed below, following the list of localities in nature where populations were sampled. Although more than 700 specimens were examined, only a small percentage was taken from the field. The vast majority were bred or cloned in the laboratory from stock collected at the localities, here listed according to the taxa or hybrids representing the populations sampled. Following the list of localities for the SIMR, MCZ, and AMNH specimens that comprise the bulk of this report, we list two localities for the four MSB specimens that had been previously reported in the literature as presumptive hybrids.

### Localities Represented

Aspidoscelis priscillae *and $F_1$ Hybrids of NM Stock*. Both the *A. uniparens* and *A. inornatus* from which the hybrids were bred and the resulting *A. priscillae* were cloned were collected at the NM localities listed below for these parental taxa. Many of the nonhybrid individuals of these taxa were raised in the laboratory also, from stock obtained at these NM localities.

Aspidoscelis priscillae *and $F_1$ Hybrids of AZ Stock*. Both the *A. uniparens* and *A. inornatus* from which the hybrids were bred and the resulting *A. priscillae* were cloned were collected at the AZ localities listed below for these parental taxa. Many of the nonhybrid individuals of these taxa were raised in the laboratory also, from stock obtained at these AZ localities.

Aspidoscelis uniparens *of NM Stock*. **New Mexico:** either *Otero County:* Alamogordo, ca. 32°52′49.5258″N, 105°57′43.2246″W in WGS 84, or *Socorro County:* vicinity of Socorro and the Rio Grande, ca. 34°3′50.2344″N, 106°52′32.9484″W, in WGS 84. As individuals from either locality were not tracked separately, each specimen is from one or the other of these two localities.

Aspidoscelis uniparens *of AZ Stock*. **Arizona:** *Cochise County:* along Arizona Highway 186 SE Willcox, ca. 32°11′43.2″N, 109°45′5.1″W at 1,268 m elev., in WGS 84.

Aspidoscelis inornatus *of NM Stock*. **New Mexico:** *Otero County:* vicinity of the city park in Alamogordo or a short walk away at ca. 32°52′47.6148″N, 105°57′53.0382″W, in WGS 84.

Aspidoscelis inornatus *of AZ Stock*. **Arizona:** *Cochise County:* along Arizona Highway 186 at 6.4 km SE Willcox at South Blue Sky Road, ca. 32°12′58.8″N, 109°46′55″W at 1,215 m elev, in WGS 84.

*MSB Localities for Presumptive Hybrids of* A. uniparens × A. inornatus. Two localities are involved for four specimens. MSB 7794–7796 were collected at **New Mexico:** *Socorro County:* transect 8 mi W and 2 mi N to 9.7 mi W and 1.3 mi N Socorro. MSB 7922 was collected at **New Mexico:** *Luna County:* approximately 0.3

mi N Nutt (by road). Our CVA (Table 8) indicated that MSB 7795 and 7796 actually represent *A. inornata* and MSB 7794 and 7922 are $F_1$ hybrids (both are males).

## Specimens Examined

*Coloration of Adult* A. priscillae *and F1 Hybrids of NM Stock*. Living *A. priscillae* included MCZ R-193485, MCZ R-193489, AMNH R-178211, and the following SIMR specimens: 17966, 17973, 18139, 18178, 18249, and 18280; hybrids were MCZ R-193460, R-193462, and AMNH R-178207.

*Coloration of Hatchling and Juvenile* A. priscillae *of NM Stock*. Living specimens included MCZ R-193494, AMNH R-178213, and the following SIMR specimens: 20142, and 20143.

*Coloration of Adult* A. priscillae *and $F_1$ Hybrids of AZ Stock*. Living *A. priscillae* included the following SIMR specimens: 18304, 18542, 18640, 18681, 18697, 18964, 18970, and 18988; the hybrids were MCZ R-194398 and R-194404.

*Coloration of Juvenile* A. priscillae *of AZ Stock*. Living specimens included SIMR 19727 and 19728.

*Coloration of Adults and Juveniles of* A. uniparens *of NM Stock*. Living specimens included AMNH R-177800 and R-178186, and SIMR specimens 20135, 20137, 20185, and 20301.

*Coloration of Adults and Hatchlings of* A. uniparens *of AZ Stock*. Living specimens included AMNH R-178199 and SIMR specimens 17149, 19364, 19402, 19439, 20954 (hatchling), and 21013 (hatchling).

*Coloration of Adult* A. inornatus *of NM Stock*. Living specimens included AMNH R-178164–178166 and SIMR specimens 17119, 17457, 17648, and notes in Cole *et al.* (2014).

*Coloration of Adult* A. inornatus *of AZ Stock*. Living specimens included SIMR 12849, 13135, 13140, 13299, 13737, and notes in Cole *et al.* (2010).

*Specimens of* A. priscillae *Used to Determine the Karyotype*. Three embryos from SIMR egg clutch M136 were used to determine the karyotype. That clutch of eggs was laid by an $F_1$ hybrid female of *A. uniparens* × *A. inornatus* of NM stock, so the embryos represented the $P_1$ generation of *A. priscillae*. The eggs were laid when multiple female hybrids occupied the enclosure, so the individual parent is not known but it was one of the following: MCZ R-193458 and R-193472 and AMNH R-177276, R-178206, and R-178207.

*Specimens of* A. priscillae *Used to Study msDNA, DNA Quantification, and Chromosome Pairing in Meiosis*. SIMR catalog numbers for all these specimens are presented in the text and illustrations where this information is presented and discussed (Figs. 4–6).

*Paratypes and Specimens of* A. priscillae *of NM Stock used in Multivariate Statistical Analyses*. MCZ R-193477, R-193484, R-193489–193491, R-193494, R-193499, R-193501, R-193504, R-193505, R-193509, R-193511, R-193512, R-193514, R-193518, R-193519, R-193521, R-193846–193855, R-194152, R-194153, R-194157–194164, R-194167, R-194296, R-194297, R-194311, R-194320, and R-194321; also AMNH R-177181–177183, R-177185–177187, R-177190–177198, R-177201, R-177204, R-177207, R-177208, R-177210–177217, R-177220, R-177221, R-177227, R-177229, R-177232, R-177234, R-177277–177283, R-177285, R-177820, R-177822–177825, R-177828–177830, R-177833, R-177835, R-177836, R-177837, R-177839, R-178212, R-178216, R-178218, R-178226, R-178228, R-178243–178247, and R-178273.

*Paratypes and Specimens of* A. priscillae *of AZ Stock Used in Multivariate Statistical Analyses*. AMNH R-178200–178204.

*Paratypes and Specimens of* A. priscillae *of NM Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. All paratypes used in multivariate analyses were also used in the

univariate analyses. In addition, the following specimens, which did not have complete characters for all 10 meristic morphological characters, were also used: MCZ R-193478–193480, R-193482–193483, R-193485–193488, R-193492–193493, R-193495–193498, R-193500, R-193502–193503, R-193506–193508, R-193510, R-193513, R-193515–193517, R-193520, R-194148–194151, R-194154–194156, R-194165–194166, R-194299, R-194310, R-194312–194319, R-194322–194328, R-194330–194340, R-194342–194348, R-194350–194351, R-194353–194386, and R-194388–194397; also AMNH R-177184, R-177188–177189, R-177199–177200, R-177202–177203, R-177205–177206, R-177209, R-177218–177219, R-177222–177226, R-177228, R-177230–177231, R-177233, R-177284, R-177821, R-177826–177827, R-177831–177832, R-177834, R-177838, R-178208–178211, R-178213–178215, R-178217, R-178219–178225, R-178227, R-178229–178242, R-178248–178272, and R-178274–178289.

*Paratypes and Specimens of* A. priscillae *of AZ Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. All paratypes used in multivariate analyses were also used in the univariate analyses. In addition, the following specimens, which did not have complete characters for all 10 meristic morphological characters, were also used: MCZ R-194102–194108 and R-194409; also AMNH R-177818–177819 and R-178200–178204.

*Specimens of $F_1$ Hybrids of* A. uniparens × A. inornatus *of NM Stock Used in Multivariate Statistical Analyses*. MCZ R-193462, R-193464, R-193468–193470, and R-193524; also AMNH R-177169–177170, R-177176–177177, and R-177276.

*Specimens of $F_1$ Hybrids of* A. uniparens × A. inornatus *of NM Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. All specimens used in multivariate analyses were also used in the univariate analyses. In addition, the following specimens, which did not have complete characters for all 10 meristic morphological characters, were also used: MCZ R-193457–193476 and R-193522–193523; also AMNH R-177167–177168, R-177171–177175, R-177178–177180, and R-178205–178207.

*Specimens of $F_1$ Hybrids of* A. uniparens × A. inornatus *of AZ Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. MCZ R-193816–193825, R-194098–194101, R-194387, R-194398, R-194402, and R-194404; also AMNH R-177266–177275 and R-177809–177817.

*Specimen of $F_1$ Hybrid of* A. uniparens *of NM Stock* × A. inornatus *of AZ Stock*. Only one was examined for this report, MCZ R-194410 (=SIMR 18782).

*Specimens of* A. uniparens *of NM Stock Used in Multivariate Statistical Analyses*. MCZ R-193826–193831, R-193833, R-194113, R-194119–194120, and R-194305; also AMNH R-177246, R-177249, R-177251, R-177253–177254, R-177807, R-178186–178187, and R-178189–178194.

*Specimens of* A. uniparens *of NM Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. All specimens used in multivariate analyses were also used in the univariate analyses. In addition, the following specimens, which did not have complete characters for all 10 meristic morphological characters, were also used: MCZ R-193832, R-193834–193835, R-194109–194112, R-194114–194118, R-194121, R-194284–194298, R-194300–194304, R-194306–194309, R-194341, R-194403, and R-194405; also AMNH R-177247–177248, R-177250, R-177252, R-177255, R-177797–177806, R-177808, R-178170–178185, and R-178188.

*Specimens of* A. uniparens *of AZ Stock Used in Multivariate Statistical Analyses*. MCZ R-193838–193840, R-193842–193843, R-194122–194123, R-194126–194127, R-194133, R-194135, R-194142, R-194144–

194145, and R-194408; also AMNH R-177258, R-178195, and R-178198–178199.

*Specimens of* A. uniparens *of AZ Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. All specimens used in multivariate analyses were also used in the univariate analyses. In addition, the following specimens, which did not have complete characters for all 10 meristic morphological characters, were also used: MCZ R-193836–193837, R-193841, R-193844–193845, R-194124–194125, R-194128–194132, 194134, R-194136–194141, R-194143, R-194146–194147, R-194329, and R-194406–194407; also AMNH R-177256–177257, R-177259–177265, R-177766–177796, and R-178196–178197.

*Specimens of* A. inornatus *of NM Stock Used in Multivariate Statistical Analyses*. MCZ R-100425, R-192214, R-192215, R-192222, R-192228, R-192230, R-192232, and R-192264; also AMNH R-177239, R-177240, R-177242, and R-178163.

*Specimens of* A. inornatus *of NM Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. All specimens used in multivariate analyses were also used in the univariate analyses. In addition, the following specimens, which did not have complete characters for all 10 meristic morphological characters, were also used: MCZ R-192220–192221, R-192223, R-192235, and R-193806–193815; also AMNH R-177236–177238, R-177241, R-177243–177245, R-178162, and R-178164–178169.

*Specimens of* A. inornatus *of AZ Stock Used in Multivariate Statistical Analyses*. MCZ R-193796–193797, R-193801–193802, R-193805, R-194399, and R-194401; also AMNH R-126871–126877, R-129205–129207, R-133219–133223, R-135033, R-148213, R-148215–148216, R-148221, R-148225, R-148229, R-148231–148232, R-148234, R-148240–148241, R-148431, and R-153166–153168.

*Specimens of* A. inornatus *of AZ Stock Used in Univariate Statistical Analyses and Recording Condition of Vertebral Stripe*. All specimens used in multivariate analyses were also used in the univariate analyses. In addition, the following specimens, which did not have complete characters for all 10 meristic morphological characters, were also used: MCZ R-193798–193800, R-193803–193804, R-194349, R-194352, and R-194400; also AMNH R-134993, R-135020, R-148211–148212, R-148214, R-148217, R-148220, R-148222, R-148224, R-148228, R-148231, R-148233, and R-148235–148237.

## APPENDIX 2

Abbreviations for morphological characters used are as follows: COS, number of circumorbital semicircle scales (total of both sides of head; following Wright and Lowe, 1967); GAB, number of dorsal granules (scales) around midbody, following Wright and Lowe (1967); FP, number of femoral pores (total of both legs); GUL, number of gular scales, following Cole *et al.* (1988); LSG, number of lateral supraocular granules (total of both sides of head, whether in one or two rows) between the supraoculars and superciliaries, counting forward from an imaginary line extended from the suture between the third and fourth supraoculars toward the superciliaries, following Walker *et al.* (1966); PAS, condition of preanal scales, in which type I means three enlarged scales, two bordering the vent and one anterior to these, type II means two enlarged scales, one bordering the vent and one anterior to it, type III means the pattern is different from both I and II; PSC, total number of scales in contact with outer perimeter of parietal and interparietal scales, following Cole *et al.* (2010); SDL-F, number of subdigital lamellae on the fourth finger (total of both sides), following Taylor *et al.* (2001); SDL-T, number of subdigital lamellae on the fourth toe (total of both sides), following Cole *et al.* (1988); SPV, number of granules (scales) between the paravertebral light stripes, following Wright and Lowe (1967); SVL, snout–vent (body) length in millimeters; TBS, number of enlarged dorsal scales around dorsal aspect of base of tail; the count is made while holding the hind legs at the hip perpendicular to the body and counting on an imaginary line along the posterior edges of the legs, but not including lateral granules on the tail.

Appendix 3. Extent of vertebral light stripe (in %) on specimens of *Aspidoscelis priscillae* and its parental taxa.

| Sample (sample size) | Nape Only | To Arms | Just Past Arms | To Midbody | Just Past Mid-body | Complete to Legs[a] |
|---|---|---|---|---|---|---|
| New Mexico (NM) *A. uniparens* (92) | 0.0 | 67.4 | 32.6 | 0.0 | 0.0 | 0.0 |
| Arizona (AZ) *A. uniparens* (88) | 0.0 | 0.0 | 3.4 | 1.1 | 0.0 | 95.4 |
| NM *A. inornatus* (30) | 0.0 | 3.3 | 0.0 | 6.7 | 0.0 | 90.0 |
| AZ *A. inornatus* (41) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| $F_1$ hybrids of NM stock (39) | 41.0 | 46.2 | 12.8 | 0.0 | 0.0 | 0.0 |
| $F_1$ hybrids of AZ stock (37) | 0.0 | 0.0 | 0.0 | 8.1 | 0.0 | 91.9 |
| NM *A. priscillae* (325) | 16.6 | 38.8 | 31.4 | 5.5 | 0.6 | 7.1 |
| AZ *A. priscillae* (15) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |

[a] Stripe may be conspicuous, subtle or pale, and may have small breaks.

Appendix 4. Loadings: Correlations between univariate characters and principal components (PCs) and between PCs and canonical variates (CVs) from multivariate analyses of *Aspidoscelis priscillae* and its progenitor species, *A. uniparens* and *A. inornatus* from New Mexico stock.

| | Character[a] | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|---|
| PC analysis[b] | | | | | | | |
| | SDL-T | −0.864 | −0.036 | −0.057 | −0.177 | 0.162 | 0.018 |
| | SPV | 0.826 | −0.123 | 0.008 | −0.146 | 0.029 | 0.316 |
| | FP | −0.773 | −0.050 | 0.124 | −0.144 | 0.359 | 0.117 |
| | COS | −0.752 | 0.137 | 0.046 | −0.209 | −0.129 | −0.348 |
| | SDL-F | −0.643 | 0.267 | 0.342 | 0.092 | −0.046 | 0.244 |
| | GUL | −0.452 | −0.271 | −0.098 | 0.816 | −0.052 | 0.057 |
| | GAB | −0.361 | −0.678 | −0.343 | −0.172 | 0.156 | 0.355 |
| | LSG | −0.303 | −0.604 | 0.079 | −0.178 | −0.675 | 0.020 |
| | TBS | 0.256 | −0.737 | 0.014 | 0.028 | 0.309 | −0.426 |
| | PSC | 0.166 | −0.333 | 0.881 | 0.025 | 0.095 | 0.055 |
| Eigenvalues | | 3.526 | 1.661 | 1.048 | 0.855 | 0.762 | 0.609 |
| Proportion of variation (%) | | 35.3 | 16.6 | 10.5 | 8.6 | 7.6 | 6.1 |
| CV analysis (CVA) | Character | CV1 | CV2 | | | | |
| | PC1 | 0.709 | 0.110 | | | | |
| | PC2 | −0.061 | 0.885 | | | | |
| | PC3 | 0.100 | 0.177 | | | | |
| | PC4 | 0.086 | −0.195 | | | | |
| | PC5 | −0.104 | −0.064 | | | | |
| | PC6 | 0.047 | −0.010 | | | | |
| Eigenvalues | | 4.215 | 0.872 | | | | |
| Proportion of intergroup variation (%) | | 82.9 | 17.1 | | | | |

[a] Univariate characters are described in Appendix 2.

[b] Loadings are not shown for PC7, PC8, PC9, and PC10 that summarized, respectively, 5.4%, 4.5%, 3.0%, and 2.5% of the meristic variation. Stepwise variable selection did not include these four components in the CVA model.

APPENDIX 5. LOADINGS: CORRELATIONS BETWEEN UNIVARIATE CHARACTERS AND PRINCIPAL COMPONENTS (PCs) AND BETWEEN PCs AND CANONICAL VARIATES (CVs) FROM MULTIVARIATE ANALYSES OF *ASPIDOSCELIS PRISCILLAE* AND ITS PROGENITOR SPECIES, *A. UNIPARENS* AND *A. INORNATUS* FROM ARIZONA STOCK.

| | Character[a] | PC1 | PC2 | PC3 | PC4 | PC8 |
|---|---|---|---|---|---|---|
| PC analysis[b] | | | | | | |
| | SDL-T | 0.939 | −0.035 | 0.027 | 0.017 | −0.008 |
| | FP | 0.864 | 0.115 | −0.042 | 0.072 | 0.021 |
| | SDL-F | 0.825 | 0.074 | 0.006 | −0.029 | 0.516 |
| | GAB | 0.712 | 0.150 | −0.185 | 0.298 | −0.056 |
| | SPV | −0.668 | 0.313 | 0.259 | −0.389 | 0.235 |
| | COS | 0.639 | 0.155 | 0.502 | −0.126 | −0.150 |
| | LSG | 0.633 | −0.000 | 0.349 | −0.414 | −0.205 |
| | GUL | −0.278 | 0.385 | 0.458 | 0.656 | 0.004 |
| | TBS | 0.141 | 0.575 | −0.657 | −0.117 | −0.113 |
| | PSC | −0.122 | 0.813 | 0.093 | −0.116 | −0.024 |
| Eigenvalues | | 4.184 | 1.304 | 1.128 | 0.891 | 0.403 |
| Proportion of variation (%) | | 41.8 | 13.0 | 11.3 | 8.9 | 4.0 |
| CV analysis (CVA) | Character | CV1 | CV2 | | | |
| | PC1 | −0.849 | −0.169 | | | |
| | PC2 | 0.006 | 0.719 | | | |
| | PC3 | 0.042 | 0.135 | | | |
| | PC4 | −0.051 | 0.388 | | | |
| | PC8 | 0.032 | −0.422 | | | |
| Eigenvalues | | 7.628 | 0.273 | | | |
| Proportion of intergroup variation (%) | | 96.5 | 3.5 | | | |

[a] Univariate characters are described in Appendix 2.

[b] Loadings are not shown for PC5, PC6, PC7, PC9, and PC10 that summarized minimal remaining variation. Stepwise variable selection did not include these five components in the CVA model.

APPENDIX 6. LOADINGS: CORRELATIONS BETWEEN UNIVARIATE CHARACTERS AND PRINCIPAL COMPONENTS (PCs) AND BETWEEN PRINCIPAL COMPONENTS AND CANONICAL VARIATES (CVs) FROM MULTIVARIATE ANALYSES OF PARTHENOGENETIC *ASPIDOSCELIS UNIPARENS* AND GONOCHORISTIC *A. INORNATUS* FROM BOTH NEW MEXICO AND ARIZONA STOCK, AND THEIR CLONED HYBRID DERIVATIVE FROM EACH STOCK, PARTHENOGENETIC *A. PRISCILLAE*.

| | Character[a] | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|---|
| PC analysis | | | | | | |
| | SDL-T | 0.930 | 0.001 | −0.090 | 0.010 | −0.096 |
| | FP | 0.798 | −0.229 | −0.027 | −0.037 | −0.155 |
| | SDL-F | 0.790 | 0.143 | −0.004 | −0.198 | −0.152 |
| | SPV | −0.783 | −0.292 | −0.011 | −0.023 | 0.290 |
| | COS | 0.743 | 0.042 | 0.047 | −0.215 | 0.213 |
| | LSG | 0.510 | −0.389 | −0.105 | 0.056 | 0.644 |
| | GAB | 0.316 | −0.694 | 0.048 | 0.379 | −0.006 |
| | PSC | −0.241 | −0.483 | −0.091 | −0.810 | −0.042 |
| | TBS | −0.173 | −0.702 | −0.258 | 0.136 | −0.389 |
| | GUL | 0.039 | −0.258 | 0.947 | −0.044 | −0.060 |
| Eigenvalues | | 3.742 | 1.586 | 0.996 | 0.911 | 0.757 |
| Proportion of variation (%) | | 37.4 | 15.9 | 10.0 | 9.1 | 7.6 |
| CV analysis | Character | CV1 | CV2 | CV3 | CV4 | CV5 |
| | PC1 | 0.684 | 0.153 | −0.134 | 0.005 | 0.087 |
| | PC2 | 0.047 | -0.720 | −0.396 | 0.387 | −0.110 |
| | PC3 | −0.037 | 0.152 | 0.082 | 0.855 | 0.037 |
| | PC4 | 0.030 | 0.164 | 0.057 | 0.114 | −0.801 |
| | PC5 | −0.069 | 0.028 | −0.084 | −0.102 | −0.064 |
| Eigenvalues | | 9.444 | 1.444 | 0.422 | 0.173 | 0.014 |
| Proportion of intergroup variation (%) | | 82.1 | 12.6 | 3.7 | 1.5 | 0.1 |

[a] Univariate characters are described in Appendix 2.

APPENDIX 7. TYPE OF PREANAL SCALES (PAS, IN %) ON SPECIMENS OF *ASPIDOSCELIS PRISCILLAE* AND ITS PARENTAL TAXA.

| Sample (sample size) | I | II | III |
|---|---|---|---|
| New Mexico (NM) *A. uniparens* (58) | 51.7 | 19.0 | 29.3 |
| Arizona (AZ) *A. uniparens* (70) | 67.1 | 12.9 | 20.0 |
| NM *A. inornatus* (22) | 90.9 | 0.0 | 9.1 |
| AZ *A. inornatus* (54) | 74.1 | 0.0 | 25.9 |
| $F_1$ hybrids of NM stock (38) | 60.5 | 5.3 | 34.2 |
| $F_1$ hybrids of AZ stock (37) | 21.6 | 51.4 | 27.0 |
| NM *A. priscillae* (159) | 35.2 | 6.3 | 58.5 |
| AZ *A. priscillae* (5) | 40.0 | 0.0 | 60.0 |

APPENDIX 8. NUMBER OF SPECIMENS (IN %) WITH SPECIFIED INTERFEMORAL SCALE COUNTS (IFS) FOR *ASPIDOSCELIS PRISCILLAE* AND ITS PARENTAL TAXA.

| Sample (sample size) | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| New Mexico (NM) *A. uniparens* (58) | 1.7 | 98.3 | 0.0 | 0.0 | 0.0 |
| Arizona (AZ) *A. uniparens* (70) | 34.3 | 65.7 | 0.0 | 0.0 | 0.0 |
| NM *A. inornatus* (21) | 0.0 | 23.8 | 61.9 | 9.5 | 4.8 |
| AZ *A. inornatus* (36) | 0.0 | 5.6 | 69.4 | 22.2 | 2.8 |
| NM *A. priscillae* (160) | 1.0 | 87.5 | 11.3 | 0.0 | 0.0 |
| AZ *A. priscillae* (5) | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |

APPENDIX 9. LOADINGS: CORRELATIONS BETWEEN UNIVARIATE CHARACTERS AND PRINCIPAL COMPONENTS (PCS) AND BETWEEN PCS AND CANONICAL VARIATES (CVS) FROM MULTIVARIATE ANALYSES OF SAMPLES OF *ASPIDOSCELIS UNIPARENS* AND *A. INORNATUS* FROM NEW MEXICO STOCK, AND THEIR $F_1$ LABORATORY HYBRIDS. THESE THREE A PRIORI GROUPS WERE USED TO DETERMINE THE MORPHOLOGICAL AFFINITIES OF FOUR FIELD-CAUGHT SPECIMENS PREVIOUSLY IDENTIFIED AS PUTATIVE HYBRIDS (WRIGHT, 1968).

| | Character[a] | PC1 | PC3 | PC4 |
|---|---|---|---|---|
| PC analysis[b] | | | | |
| | SDL-T | −0.881 | 0.044 | 0.181 |
| | SPV | 0.755 | −0.332 | −0.129 |
| | FP | −0.756 | 0.030 | 0.390 |
| | COS | −0.728 | 0.385 | −0.001 |
| | GUL | −0.700 | −0.008 | −0.023 |
| | GAB | −0.675 | −0.550 | −0.075 |
| | LSG | −0.543 | −0.276 | −0.593 |
| | SDL-F | −0.486 | 0.297 | −0.446 |
| | TBS | −0.280 | −0.619 | 0.269 |
| | PSC | 0.158 | 0.232 | 0.139 |
| Eigenvalues | | 4.031 | 1.165 | 0.850 |
| Proportion of variation (%) | | 40.3 | 11.6 | 8.5 |
| CV analysis (CVA) | Character | CV1 | CV2 | |
| | PC1 | −0.741 | 0.394 | |
| | PC3 | 0.048 | 0.999 | |
| | PC4 | 0.074 | −0.018 | |
| Eigenvalues | | 9.330 | 0.531 | |
| Proportion of intergroup variation (%) | | 94.6 | 5.4 | |

[a] Univariate characters are described in Appendix 2.

[b] Loadings are shown for the three PCs selected as having discrimination value by the CVA model. PCs not included in the CVA and the proportion of variation represented by each were PC2, 15.9%; PC5, 6.7%; PC6, 6.1%; PC7, 4.1%; PC8, 3.0%; PC9, 2.0%, and PC10, 1.7%.

Appendix 10. Loadings: Correlations between univariate characters and principal components (PCs) from a PC analysis (PCA) of *Aspidoscelis priscillae* and its progenitor species, *A. uniparens* and *A. inornatus*, from New Mexico stock. For each sample, $N = 12$.

| New Mexico comparisons | Character[a] | PC1 | PC2 | PC3 |
|---|---|---|---|---|
| PCA[b] | | | | |
| | SDL-T | −0.909 | 0.046 | 0.041 |
| | SPV | 0.856 | 0.186 | −0.176 |
| | COS | −0.839 | −0.066 | 0.067 |
| | FP | −0.822 | 0.059 | 0.243 |
| | GAB | −0.721 | 0.150 | −0.445 |
| | CUL | −0.640 | −0.232 | −0.244 |
| | SDL-F | −0.597 | −0.035 | 0.540 |
| | LSC | −0.438 | 0.564 | −0.264 |
| | PSC | 0.213 | 0.597 | 0.718 |
| | TBS | −0.077 | 0.850 | −0.271 |
| Eigenvalues | | 4.468 | 1.518 | 1.304 |
| Proportion of variation (%) | | 44.7 | 15.2 | 13.0 |

[a] Univariate characters are described in Appendix 2.

[b] Loadings are not shown for PC4, PC5, PC6, PC7, PC8, PC9, and PC10 that summarized, respectively, 8.5%, 6.8%, 5.1%, 2.6%, 2.1%, 1.5%, and 0.9% of the meristic variation.

Loadings: Correlations between univariate characters and principal components (PCs) from a PC analysis (PCA) of *Aspidoscelis uniparens*, *A. inornatus*, and $F_1$ hybrids from Arizona stock. For each sample, $N = 19$.

| Arizona comparisons | Character | PC1 | PC2 | PC3 |
|---|---|---|---|---|
| PCA[a] | | | | |
| | SDL-T | 0.938 | −0.026 | −0.086 |
| | FP | 0.871 | −0.017 | 0.084 |
| | SDL-F | 0.822 | 0.064 | −0.085 |
| | LSC | 0.677 | 0.154 | 0.245 |
| | SPV | −0.697 | 0.289 | 0.191 |
| | COS | 0.664 | 0.474 | −0.140 |
| | GAB | 0.477 | −0.172 | 0.411 |
| | CUL | −0.235 | 0.567 | 0.410 |
| | TBS | −0.005 | −0.746 | −0.147 |
| | PSC | −0.049 | 0.388 | −0.757 |
| Eigenvalues | | 3.983 | 1.394 | 1.070 |
| Proportion of variation (%) | | 39.8 | 13.9 | 10.7 |

[a] Loadings are not shown for PC4, PC5, PC6, PC7, PC8, PC9, and PC10 that summarized, respectively, 9.0%, 7.7%, 7.1%, 4.6%, 3.4%, 2.4%, and 1.2% of the meristic variation.

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Photo on the front cover:

*Aspidoscelis priscillae*: SIMR 23270, young representative of the $F_7$ generation (snout-vent length of 64 mm, 224 days of age) photographed on 16 June 2017 by William B. Neaves.

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Museum of Comparative Zoology
Harvard University
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